LUTZOMYIA LONGIPALPIS POLYPEPTIDES AND METHODS OF USE

PRIORITY CLAIM

This application claims the benefit of U.S. Provisional Application No. 60/422,303, filed October 29, 2002, which is incorporated herein by reference.

FIELD

The disclosure relates to proteins substantially purified from Lutzomyia longipalpis (*Lu. longipalpis*) sand fly salivary glands, or recombinant vectors expressing these proteins, and to an immune response produced to these proteins. This disclosure also relates to the production of an immune response that affects survival of Leishmania.

BACKGROUND

Leishmaniasis is a group of diseases caused by protozoa of the genus *Leishmania* and affect many millions of people worldwide. In humans, infection with the parasite manifests either as a cutaneous disease caused mainly by *L. major, L. tropica*, and *L. mexicana*; as a mucocutaneous disease caused mainly by *L. brasiliensis*; or as a visceral disease caused mainly by *L. donovani* and *L. chagasi*. In canids, Leishmania infections manifest as a visceral disease that can result in high death rates.

All leishmanial diseases are transmitted to their vertebrate hosts by phlebotomine sand flies, which acquire the pathogen by feeding on infected hosts and transmit them by regurgitating the parasite at the site of a subsequent blood meal (Killick-Kendrick, Biology of *Leishmania* in phlebotomine sand flies. In Biology of the Kinetoplastida. W. Lumsden and D. Evans, editors. Academic Press, New York. 395, 1979).

While obtaining a blood meal, sand flies salivate into the host's skin. This saliva contains anticlotting, antiplatelet, and vasodilatory compounds that increase the hemorrhagic pool where sand flies feed (Ribeiro *et al., Comp. Biochem. Physiol.* 4:683, 1986; Charlab *et al., Proc. Natl. Acad. Sci. USA.* 26:15155, 1999). Some of these components are additionally immunomodulatory. For example, the New World sand fly *Lutzomyia longipalpis* contains the 6.5 kDa peptide, maxadilan, which is the most potent vasodilator known (Lerner *et al., J. Biol. Chem.* 17:11234, 1991). Maxadilan additionally has immunosuppressive activities of its own (Qureshi *et al., Am. J. Trop. Med. Hyg.* 6:665, 1996), as do many persistent vasodilators such as prostaglandin E_2 (Makoul *et al., J. Immunol.* 134:2645, 1985; Santoli and Zurier, *J. Immunol.* 143:1303, 1989; Stockman and Mumford, *Exp. Hematol.* 2:65, 1974) and calcitonin gene-related peptide (Nong *et al., J. Immunol.* 1:45, 1989). Old World sand flies do not have maxadilan but instead use AMP and adenosine as vasodilators (Ribeiro *et al., J. Exp. Biol.* 11:1551, 1999). Adenosine is also an immunomodulatory component, promoting the production of IL-10 and suppressing TNF-α and IL-12 in mice (Hasko *et al., J. Immunol.* 10:4634, 1996; Webster, *Asian Pac. J. Allergy Immunol.* 2:311, 1984; Hasko *et al.,*

FASEB J. 14:2065, 2000). Despite what is known about the role of sand fly saliva and disease transmission, much remains unknown, and an effective vaccine does not exist. Thus, there is a need for agents that can be used to induce an immune response to the organisms that cause leishmaniasis.

SUMMARY

The present disclosure relates to salivary proteins from sand fly vectors of *Lutzomyia longipalpis* (*Lu. longipalpis*) and the nucleic acids that encode these proteins. Methods of producing an immune response in a subject are also disclosed.

Substantially purified salivary *Lu. longipalpis* polypeptides are disclosed herein. Also disclosed are polynucleotides encoding the *Lu. longipalpis* polypeptides.

Methods are disclosed for inducing an immune response using a therapeutically effective amount of a substantially purified salivary *Lu. longipalpis* polypeptide as disclosed herein, or the polynucleotide encoding a *Lu. longipalpis* polypeptides disclosed herein.

In another embodiment methods are disclosed herein for inhibiting the symptoms of a *Leishmania* infection or for preventing a *Leishmania* infection in a subject. The methods include administering to the subject a therapeutically effective amount of a *Lu. longipalpis* polypeptide, or a polynucleotide encoding a *Lu. longipalpis* polypeptide. In two non-limiting examples, more than one *Lu. longipalpis* polypeptide can be administered, or at least one *Lu. longipalpis* polypeptide in conjunction with a *P. ariasi* or *P. perniciosus* polypeptide.

Also disclosed herein are methods of diagnosing *Leishmania* infection in a subject. The methods include contacting a solid substrate comprising at least three, six, or ten *Lu. longipalpis* polypeptides, or an immunogenic fragment thereof, contacting the solid substrate with a sample obtained from the subject and detecting binding of a component of the sample to at least one polypeptide on the solid substrate. Detection of binding of the component to the substrate indicates that the subject is infected with *Leishmania*.

Pharmaceutical compositions are disclosed including a pharmaceutically acceptable carrier and a *Lu. longipalpis* polypeptide.

The foregoing and other features and advantages will become more apparent from the following detailed description of several embodiments, which proceeds with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a set of bar graphs showing the levels of antibodies against *Lutzomyia longipalpis* (*Lu. longipalpis*) saliva in sera of individuals. Human sera were obtained at time 0 (negative anti-*Leishmania* serology (S^-) or negative DTH (DTH^-)) and 6 months later (positive anti-*Leishmania* serology (S^+) or positive anti-*Leishmania* DTH (DTH^+)). ELISA was performed with these sera using salivary gland sonicate of the sand fly *Lu. longipalpis*. FIG. 1A is a bar graph of anti-saliva IgG levels in individuals who converted from $S^- \rightarrow S^+$ and those who converted from DTH^- to DTH^+. FIG. 1B is a bar graph of anti-saliva IgE levels in the individuals described in FIG. 1A. FIG. 1C is a

bar graph of anti-saliva IgG1 levels in the individuals described in FIG. 1A. FIG. 1D is a bar graph of anti-saliva IgG4 levels in the individuals described in FIG. 1A. The non-parametric paired Wilcoxon test was used to compare levels of anti-*Lu. longipalpis* saliva antibodies at time 0 and after 6 months. *P* value < 0.05 was established as the significance level.

FIG. 2 is a set of two digital images and a bar graph showing salivary proteins recognized by Western blot analysis. FIGS. 2A and 2B are digital images of a Western blot of *Lu. longipalpis* salivary proteins reacted to human sera of individuals who converted from $S^- \rightarrow S^+$ to *Leishmania* (lanes 1–6) or from $DTH^- \rightarrow DTH^+$ to *Leishmania* (lanes 7–14). Symbols: –, time 0; +, 6 months. FIG. 2C is a bar graph of the frequency of salivary proteins recognized by sera of 13 individuals who converted from $DTH^- \rightarrow DTH^+$ to *Leishmania*. The x-axis shows the different *Lu. longipalpis* salivary proteins (labeled by the approximate molecular weight) recognized by Western blot analysis, while the y-axis indicates the number of human sera recognizing a particular salivary protein.

SEQUENCE LISTING

The nucleic and amino acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, and three letter code for amino acids, as defined in 37 C.F.R. 1.822. Only one strand of each nucleic acid sequence is shown, but the complementary strand is understood as included by any reference to the displayed strand. In the accompanying sequence listing:

SEQ ID NO: 1 is the amino acid sequence of LJL34.
SEQ ID NO: 2 is the nucleic acid sequence of LJL34.
SEQ ID NO: 3 is the amino acid sequence of LJL18.
SEQ ID NO: 4 is the nucleic acid sequence of LJL18.
SEQ ID NO: 5 is the amino acid sequence of LJS193.
SEQ ID NO: 6 is the nucleic acid sequence of LJS193.
SEQ ID NO: 7 is the amino acid sequence of LJS201.
SEQ ID NO: 8 is the nucleic acid sequence of LJS201.
SEQ ID NO: 9 is the amino acid sequence of LJL13.
SEQ ID NO: 10 is the nucleic acid sequence of LJL13.
SEQ ID NO: 11 is the amino acid sequence of LJL23.
SEQ ID NO: 12 is the nucleic acid sequence of LJL23.
SEQ ID NO: 13 is the amino acid sequence of LJM10.
SEQ ID NO: 14 is the nucleic acid sequence of LJM10.
SEQ ID NO: 15 is the amino acid sequence of LJL143.
SEQ ID NO: 16 is the nucleic acid sequence of LJL143.
SEQ ID NO: 17 is the amino acid sequence of LJS142.
SEQ ID NO: 18 is the nucleic acid sequence of LJS142.

SEQ ID NO: 19 is the amino acid sequence of LJL17.
SEQ ID NO: 20 is the nucleic acid sequence of LJL17.
SEQ ID NO: 21 is the amino acid sequence of LJM06.
SEQ ID NO: 22 is the nucleic acid sequence of LJM06.
SEQ ID NO: 23 is the amino acid sequence of LJM17.
SEQ ID NO: 24 is the nucleic acid sequence of LJM17.
SEQ ID NO: 25 is the amino acid sequence of LJL04.
SEQ ID NO: 26 is the nucleic acid sequence of LJL04.
SEQ ID NO: 27 is the amino acid sequence of LJM114.
SEQ ID NO: 28 is the nucleic acid sequence of LJM114.
SEQ ID NO: 29 is the amino acid sequence of LJM111.
SEQ ID NO: 30 is the nucleic acid sequence of LJM111.
SEQ ID NO: 31 is the amino acid sequence of LJM78.
SEQ ID NO: 32 is the nucleic acid sequence of LJM78.
SEQ ID NO: 33 is the amino acid sequence of LJS238.
SEQ ID NO: 34 is the nucleic acid sequence of LJS238.
SEQ ID NO: 35 is the amino acid sequence of LJS169.
SEQ ID NO: 36 is the nucleic acid sequence of LJS169.
SEQ ID NO: 37 is the amino acid sequence of LJL11.
SEQ ID NO: 38 is the nucleic acid sequence of LJL11.
SEQ ID NO: 39 is the amino acid sequence of LJL08.
SEQ ID NO: 40 is the nucleic acid sequence of LJL08.
SEQ ID NO: 41 is the amino acid sequence of LJS105.
SEQ ID NO: 42 is the nucleic acid sequence of LJS105.
SEQ ID NO: 43 is the amino acid sequence of LJL09.
SEQ ID NO: 44 is the nucleic acid sequence of LJL09.
SEQ ID NO: 45 is the amino acid sequence of LJL38.
SEQ ID NO: 46 is the nucleic acid sequence of LJL38.
SEQ ID NO: 47 is the amino acid sequence of LJM04.
SEQ ID NO: 48 is the nucleic acid sequence of LJM04.
SEQ ID NO: 49 is the amino acid sequence of LJM26.
SEQ ID NO: 50 is the nucleic acid sequence of LJM26.
SEQ ID NO: 51 is the amino acid sequence of LJS03.
SEQ ID NO: 52 is the nucleic acid sequence of LJS03.
SEQ ID NO: 53 is the amino acid sequence of LJS192.
SEQ ID NO: 54 is the nucleic acid sequence of LJS192.
SEQ ID NO: 55 is the amino acid sequence of LJM19.
SEQ ID NO: 56 is the nucleic acid sequence of LJM19.
SEQ ID NO: 57 is the amino acid sequence of LJL138.

SEQ ID NO: 58 is the nucleic acid sequence of LJL138.
SEQ ID NO: 59 is the amino acid sequence of LJL15.
SEQ ID NO: 60 is the nucleic acid sequence of LJL15.
SEQ ID NO: 61 is the amino acid sequence of LJL91.
SEQ ID NO: 62 is the nucleic acid sequence of LJL91.
SEQ ID NO: 63 is the amino acid sequence of LJM11.
SEQ ID NO: 64 is the nucleic acid sequence of LJM11.
SEQ ID NO: 65 is the amino acid sequence of LJS138.
SEQ ID NO: 66 is the nucleic acid sequence of LJS138.
SEQ ID NO: 67 is the amino acid sequence of LJL124.
SEQ ID NO: 68 is the nucleic acid sequence of LJL124.
SEQ ID NO: 69 is the amino acid sequence of LJL35.
SEQ ID NO: 70 is the nucleic acid sequence of LJL35.
SEQ ID NO: 71 is an oligonucleotide primer.
SEQ ID NO: 72 is an oligonucleotide primer.
SEQ ID NO: 73 is an oligonucleotide primer.

DETAILED DESCRIPTION

I. Abbreviations

AAV	adeno-associated virus
AcNPV	Autographa California Nuclear Polyhedrosis Virus
alum	aluminum phosphate or aluminum hydroxide
BCG	Bacillus Calmette Guerin
BLAST	Basic Local Alignment Search Tool
BSA	bovine serum albumin
CAV	canine adenovirus
CDR	complementarity determining region
CHV	canine herpes virus
CMV	cytomegalovirus
CTL	cytotoxic T lymphocyte
DMRIE	N-(2-hydroxyethyl)-N,N-diméthyl-2,3-bis(tetradecyloxy)-1-propanammonium
DOPE	dioleoyl-phosphatidyl-ethanolamine
DTH	delayed type hypersensitivity
fMLP	N-formyl-methionyl-leucyl-phenylalanine
GM-CSF	granulocyte-macrophage colony stimulating factor
H	heavy chains

HLB	hydrophile-lipophile balance
ID	intradermal
IM	intramuscular
ISS	immunostimulating sequence
KLH	keyhole limpet hemocyanin
L	light chains
LB	Luria broth
Lu. longipalpis	*Lutzomyia logipalpis*
MVA	Modified Vaccinia virus Ankara
OFR	open reading frame
P. ariasi	*Phlebotomus ariasi*
PCR	polymerase chain reaction
polyA	polyadenylation signal
P. papatasi	*Phlebotomus papatasi*
PVDF	polyvinylidene difluoride
SC	subcutaneous
SCA	Single chain antibody
sFv	single-chain antigen binding proteins
SGH	salivary gland homogenate
SPGA	sucrose phosphate glutamate albumin
tPA	tissue plasminogen activator
V_H	variable region of the heavy chain
V_L	variable region of the light chain
VL	visceral leishmaniasis

II. Terms

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Benjamin Lewin, *Genes V*, published by Oxford University Press, 1994 (ISBN 0-19-854287-9); Kendrew *et al.* (eds.), *The Encyclopedia of Molecular Biology*, published by Blackwell Science Ltd., 1994 (ISBN 0-632-02182-9); and Robert A. Meyers (ed.), *Molecular Biology and Biotechnology: a Comprehensive Desk Reference*, published by VCH Publishers, Inc., 1995 (ISBN 1-56081-569-8).

In order to facilitate review of the various embodiments of the disclosure, the following explanations of specific terms are provided:

Amplification of a nucleic acid molecule (for example, a DNA or RNA molecule): A technique that increases the number of copies of a nucleic acid molecule in a specimen. An example of amplification is the polymerase chain reaction, in which a biological sample collected from a

subject is contacted with a pair of oligonucleotide primers, under conditions that allow for the hybridization of the primers to a nucleic acid template in the sample. The primers are extended under suitable conditions, dissociated from the template, and then re-annealed, extended, and dissociated to amplify the number of copies of the nucleic acid. The product of amplification may be characterized by electrophoresis, restriction endonuclease cleavage patterns, oligonucleotide hybridization or ligation, and/or nucleic acid sequencing using standard techniques. Other examples of amplification include strand displacement amplification, as disclosed in U.S. Patent No. 5,744,311; transcription-free isothermal amplification, as disclosed in U.S. Patent No. 6,033,881; repair chain reaction amplification, as disclosed in WO 90/01069; ligase chain reaction amplification, as disclosed in EP 0320308; gap filling ligase chain reaction amplification, as disclosed in U.S. Patent No. 5,427,930; and NASBA™ RNA transcription-free amplification, as disclosed in U.S. Patent No. 6,025,134.

Antibody: immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, for instance, molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen.

A naturally occurring antibody (for example, IgG, IgM, IgD) includes four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. However, it has been shown that the antigen-binding function of an antibody can be performed by fragments of a naturally occurring antibody. Thus, these antigen-binding fragments are also intended to be designated by the term "antibody." Specific, non-limiting examples of binding fragments encompassed within the term antibody include (i) an Fab fragment consisting of the V_L, V_H, CL, and CH1 domains; (ii) an Fd fragment consisting of the V_H and CH1 domains; (iii) an Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (iv) a dAb fragment (Ward *et al.*, *Nature* 341:544-546, 1989) which consists of a V_H domain; (v) an isolated complimentarity determining region (CDR); and (vi) an $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region.

Immunoglobulins and certain variants thereof are known and many have been prepared in recombinant cell culture (for example, see U.S. Patent No. 4,745,055; U.S. Patent No. 4,444,487; WO 88/03565; EP 0256654; EP 0120694; EP 0125023; Faoulkner *et al.*, *Nature* 298:286, 1982; Morrison, *J. Immunol.* 123:793, 1979; Morrison *et al.*, *Ann Rev. Immunol* 2:239, 1984).

Animal: Living multi-cellular vertebrate organisms, a category that includes, for example, mammals and birds. The term mammal includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects, such as dogs.

Conservative variants: Conservative amino acid substitutions are those substitutions that do not substantially affect or decrease an activity or antigenicity of the *Lu. longipalpis* polypeptide. Specific, non-limiting examples of a conservative substitution include the following examples:

Original Residue	Conservative Substitutions
Ala	Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
His	Asn; Gln
Ile	Leu, Val
Leu	Ile; Val
Lys	Arg; Gln; Glu
Met	Leu; Ile
Phe	Met; Leu; Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp; Phe
Val	Ile; Leu

The term conservative variation also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid, provided that antibodies raised to the unsubstituted polypeptide also essentially immunoreact with the substituted polypeptide, or that an immune response can be generated against the substituted polypeptide that is similar to the immune response against the unsubstituted polypeptide. Thus, in one embodiment, non-conservative substitutions are those that reduce an activity or antigenicity.

cDNA (complementary DNA): A piece of DNA lacking internal, non-coding segments (introns) and expression control sequences. cDNA is synthesized in the laboratory by reverse transcription from messenger RNA extracted from cells.

Degenerate variant: A polynucleotide encoding a *Lu. longipalpis* polypeptide that includes a sequence that is degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the *Lu. longipalpis* polypeptide encoded by the nucleotide sequence is unchanged.

Delayed-type hypersensitivity (DTH): An immune reaction in which T cell-dependent macrophage activation and inflammation cause tissue injury. A DTH reaction to the subcutaneous injection of antigen is often used as an assay for cell-mediated immunity.

Epitope: An antigenic determinant. These are particular chemical groups or peptide sequences on a molecule that are antigenic, for instance, that elicit a specific immune response. An antibody specifically binds a particular antigenic epitope on a polypeptide. Specific, non-limiting examples of an epitope include a tetra- to penta-peptide sequence in a polypeptide, a tri- to penta-glycoside sequence in a polysaccharide. In the animal most antigens will present several or even many antigenic determinants simultaneously. Such a polypeptide may also be qualified as an immunogenic polypeptide and the epitope may be identified as described further.

Expression Control Sequences: Nucleic acid sequences that control and regulate the expression of a nucleic acid sequence, such as a heterologous nucleic acid sequence, to which it is operably linked. Expression control sequences are operably linked to a nucleic acid sequence when the expression control sequences control and regulate the transcription and, as appropriate, translation of the nucleic acid sequence. Thus expression control sequences can include appropriate promoters, enhancers, transcription terminators, polyA signals, a start codon (for instance, ATG) in front of a protein-encoding polynucleotide sequence, splicing signal for introns, maintenance of the correct reading frame of that gene to permit proper translation of mRNA, and stop codons. The term "control sequences" is intended to include, at a minimum, components whose presence can influence expression, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences. Expression control sequences can include a promoter.

A promoter is a minimal sequence sufficient to direct transcription of a nucleic acid. Promoters may be cell-type specific or tissue specific. A promoter includes necessary nucleic acid sequences near the start site of transcription, such as, in the case of a polymerase II type promoter, a TATA element. A promoter also optionally includes distal enhancer or repressor elements which can be located as much as several thousand base pairs from the start site of transcription. Both constitutive and inducible promoters are included (see for example, Bitter *et al., Methods in Enzymology* 153:516-544, 1987).

For example, when cloning in bacterial systems, inducible promoters such as pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac-hybrid promoter) and the like may be used. In one embodiment, when cloning in mammalian cell systems, promoters derived from the genome of mammalian cells (for example, metallothionein promoter) or from mammalian viruses (for example, the retrovirus long terminal repeat; the adenovirus late promoter; the vaccinia virus 7.5K promoter) can be used. Promoters produced by recombinant DNA or synthetic techniques may also be used to provide for transcription of the nucleic acid sequences. A polynucleotide can be inserted into an expression vector that contains a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as well as specific nucleic acid sequences that allow phenotypic selection of the transformed cells. In one embodiment, the promoter is a cytomegalovirus promoter.

Host cells: Cells in which a vector can be propagated and its DNA expressed. The cell may be prokaryotic or eukaryotic. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used. Also includes the cells of the subject.

Immune response: A response of a cell of the immune system, such as a B cell, T cell, or monocyte, to a stimulus. In one embodiment, the response is specific for a particular antigen (an "antigen-specific response"). The response can also be a non-specific response (not targeted specifically to salivary polypeptides) such as production of lymphokines. In one embodiment, an

immune response is a T cell response, such as a CD4+ response or a CD8+ response. In another embodiment, the response is a Th1 (a subset of helper T cells) response. In yet another embodiment, the response is a B cell response, and results in the production of specific antibodies.

Immunogenic polypeptide: A polypeptide which comprises an allele-specific motif, an epitope or other sequence such that the polypeptide will bind an MHC molecule and induce a cytotoxic T lymphocyte ("CTL") response, and/or a B cell response (for example, antibody production), and/or T-helper lymphocyte response, and/or a delayed type hypersensitivity (DTH) response against the antigen from which the immunogenic polypeptide is derived.

In one embodiment, immunogenic polypeptides are identified using sequence motifs or other methods known in the art. Typically, algorithms are used to determine the "binding threshold" of polypeptides to select those with scores that give them a high probability of binding at a certain affinity and will be immunogenic. The algorithms are based either on the effects on MHC binding of a particular amino acid at a particular position, the effects on antibody binding of a particular amino acid at a particular position, or the effects on binding of a particular substitution in a motif-containing polypeptide. Within the context of an immunogenic polypeptide, a "conserved residue" is one which appears in a significantly higher frequency than would be expected by random distribution at a particular position in a polypeptide. In one embodiment, a conserved residue is one where the MHC structure may provide a contact point with the immunogenic polypeptide.

Immunogenic composition: A composition that, when administered to a subject induces an immune response to a *Lu. longipalpis* salivary polypeptide. In one embodiment, in particular a positive DTH response.

Isolated: An "isolated" biological component (such as a nucleic acid or protein or organelle) has been substantially separated or purified away from other biological components in the cell of the organism in which the component naturally occurs, for instance, other chromosomal and extra-chromosomal DNA and RNA, proteins, and organelles. Nucleic acids and proteins that have been "isolated" include nucleic acids and proteins purified by standard purification methods. The term also embraces nucleic acids and proteins prepared by recombinant technology as well as chemical synthesis.

Label: A detectable compound or composition that is conjugated directly or indirectly to another molecule to facilitate detection of that molecule. Specific, non-limiting examples of labels include fluorescent tags, enzymatic linkages, and radioactive isotopes.

Leishmaniasis: A parasitic disease spread by the bite of infected sand flies. The trypanosomatid parasite of the genus *Leishmania* is the etiological agent of a variety of disease manifestations, which are collectively known as leishmaniasis. Leishmaniasis is prevalent through out the tropical and sub-tropical regions of Africa, Asia, the Mediterranean, Southern Europe (old world), and South and Central America (new world). The old world species are transmitted by the sand fly vector *Phlebotomus sp*. Humans, wild animals and domestic animals (such as dogs) are known to be targets of these sand flies and to act as reservoir hosts or to develop leishmaniasis.

Cutaneous leishmaniasis starts as single or multiple nodules that develop into ulcers in the skin at the site of the bite. The chiclero ulcer typically appears as a notch-like loss of tissue on the ear lobe. The incubation period ranges from days to months, even a year in some cases. The sores usually last months to a few years, with most cases healing on their own. The mucocutaneous type can develop into erosive lesions in the nose, mouth, or throat and can lead to severe disfigurement. Visceral leishmaniasis often has fever occurring in a typical daily pattern, abdominal enlargement with pain, weakness, widespread swelling of lymph nodes, and weight loss, as well as superimposed infections because of a weakened immune system. Visceral leishmaniasis (VL) can result in high death rates. The onset of symptoms can be sudden, but more often tends to be insidious.

***Lutzomyia longipalpis* (*Lu. longipalpis*):** A species of sand fly endogenous to the New World (South and Central America). This sand fly is the principal vector of American visceral leishmaniasis, a potentially fatal disease that primarily affects children in several countries of South and Central America.

Lymphocytes: A type of white blood cell that is involved in the immune defenses of the body. There are two main types of lymphocytes: B cells and T cells.

Mammal: This term includes both human and non-human mammals. Similarly, the term "subject" includes both human and veterinary subjects.

Oligonucleotide: A linear polynucleotide sequence of up to about 100 nucleotide bases in length.

Open reading frame (ORF): A nucleic acid sequence having a series of nucleotide triplets (codons), starting with a start codon and ending with a stop codon, coding for amino acids without any internal termination codons. These sequences are usually translatable into a polypeptide.

Operably linked: A first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein-coding regions, in the same reading frame.

Polypeptide Modifications: *Lu. longipalpis* polypeptides include synthetic embodiments of polypeptides described herein. In addition, analogues (non-peptide organic molecules), derivatives (chemically functionalized peptide molecules obtained starting with the disclosed polypeptide sequences) and variants (homologs) of these proteins can be utilized in the methods described herein. Each polypeptide of the disclosure is comprised of a sequence of amino acids, which may be either L- and/or D- amino acids, naturally occurring and otherwise.

Polypeptides may be modified by a variety of chemical techniques to produce derivatives having essentially the same activity as the unmodified polypeptides, and optionally having other desirable properties. For example, carboxylic acid groups of the protein, whether carboxyl-terminal or side chain, may be provided in the form of a salt of a pharmaceutically-acceptable cation or esterified to form a C_1-C_{16} ester, or converted to an amide of formula NR_1R_2 wherein R_1 and R_2 are

each independently H or C_1-C_{16} alkyl, or combined to form a heterocyclic ring, such as a 5- or 6-membered ring. Amino groups of the peptide, whether amino-terminal or side chain, may be in the form of a pharmaceutically-acceptable acid addition salt, such as the HCl, HBr, acetic, benzoic, toluene sulfonic, maleic, tartaric, and other organic salts, or may be modified to C_1-C_{16} alkyl or dialkyl amino or further converted to an amide.

Hydroxyl groups of the peptide side chains may be converted to C_1-C_{16} alkoxy or to a C_1-C_{16} ester using well-recognized techniques. Phenyl and phenolic rings of the peptide side chains may be substituted with one or more halogen atoms, such as fluorine, chlorine, bromine, or iodine, or with C_1-C_{16} alkyl, C_1-C_{16} alkoxy, carboxylic acids and esters thereof, or amides of such carboxylic acids. Methylene groups of the peptide side chains can be extended to homologous C_2-C_4 alkylenes. Thiols can be protected with any one of a number of well-recognized protecting groups, such as acetamide groups. Those skilled in the art will also recognize methods for introducing cyclic structures into the peptides of this disclosure to select and provide conformational constraints to the structure that result in enhanced stability.

Peptidomimetic and organomimetic embodiments are envisioned, whereby the three-dimensional arrangement of the chemical constituents of such peptido- and organomimetics mimic the three-dimensional arrangement of the peptide backbone and component amino acid side chains, resulting in such peptido- and organomimetics of a *L. longipalpis* polypeptide having measurable or enhanced ability to generate an immune response. For computer modeling applications, a pharmacophore is an idealized, three-dimensional definition of the structural requirements for biological activity. Peptido- and organomimetics can be designed to fit each pharmacophore with current computer modeling software (using computer assisted drug design or CADD). See Walters, "Computer-Assisted Modeling of Drugs," Klegerman & Groves (eds.), 1993, *Pharmaceutical Biotechnology*, Interpharm Press: Buffalo Grove, IL, pp. 165-174 and *Principles of Pharmacology* Munson (ed.) 1995, Ch. 102, for descriptions of techniques used in CADD. Also included are mimetics prepared using such techniques.

Pharmaceutically acceptable vehicles or excipients: The pharmaceutically acceptable vehicles or excipients of use are conventional. *Remington's Pharmaceutical Sciences*, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of the polypeptides, plasmids, viral vectors herein disclosed.

In general, the nature of the vehicle or excipient will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, freeze-dried pastille, powder, pill, tablet, or capsule forms), conventional non-toxic solid vehicles or excipients can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically neutral vehicles or excipients, immunogenic compositions to be administered can contain minor amounts of non-toxic

auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

Phlebotomus ariasi (P. ariasi): A species of Phlebotomus (sand flies) genus endogenous to the Old World, in particular to southern Europe and Mediterranean countries, more particularly to Spain and France. This sand fly is a proven vector of visceral leishmaniasis. *P. ariasi* is a member of the subgenera of Phlebotomus Larroussius.

Phlebotomus perniciosus (P. perniciosus): A species of Phlebotomus (sand flies) genus endogenous to the Old World, in particular to southern Europe, and Mediterranean countries, more particularly to France, Italy, Greece, Morocco, and Spain. This sand fly is a proven vector of the visceral leishmaniasis. *P. perniciosus* is a member of the subgenera of Phlebotomus Larroussius.

Polynucleotide: The term polynucleotide or nucleic acid sequence refers to a polymeric form of nucleotide at least 10 bases in length, thus including oligonucleotides and genes. A recombinant polynucleotide includes a polynucleotide that is not immediately contiguous with both of the coding sequences with which it is immediately contiguous (one on the 5' end and one on the 3' end) in the naturally occurring genome of the organism from which it is derived. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (for example, a cDNA) independent of other sequences. The polynucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single -and double -stranded forms of DNA.

Polypeptide: Any chain of amino acids, regardless of length (thus encompassing oligopeptides, peptides, and proteins) or post-translational modification (for example, glycosylation, phosphorylation, or acylation). A polypeptide encompasses also the precursor, as well as the mature protein. In one embodiment, the polypeptide is a polypeptide isolated from *Lu. longipalpis*, or encoded by a nucleic acid isolated from *Lu. longipalpis*, such as the *Lu. longipalpis* polypeptides disclosed herein.

Probes and primers: A probe comprises an isolated polynucleotide attached to a detectable label or reporter molecule. Primers are short polynucleotides. In one embodiment, polynucleotides are 15 nucleotides or more in length. Primers may be annealed to a complementary target DNA strand by nucleic acid hybridization to form a hybrid between the primer and the target DNA strand, and then extended along the target DNA strand by a DNA polymerase enzyme. Primer pairs can be used for amplification of a nucleic acid sequence, for example, by the polymerase chain reaction (PCR) or other nucleic-acid amplification methods known in the art. One of skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, for example, a primer comprising 20 consecutive nucleotides will anneal to a target with a higher specificity than a corresponding primer of only 15 nucleotides. Thus, in order to obtain greater specificity, probes and primers may be selected that comprise at least 15, 20, 25, 30, 35, 40, 50 or more consecutive nucleotides.

Protein Purification: The *Lu. longipalpis* polypeptides disclosed herein can be purified by any of the means known in the art. See, for example, *Guide to Protein Purification*, Deutscher (ed.), *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982. Substantial purification denotes purification from other proteins or cellular components. A substantially purified protein is at least 60%, 70%, 80%, 90%, 95%, or 98% pure. Thus, in one specific, non-limiting example, a substantially purified protein is 90% free of other proteins or cellular components.

Purified: The term purified does not require absolute purity; rather, it is intended as a relative term. Thus, for example, a purified polypeptide preparation is one in which the polypeptide is more enriched than the polypeptide is in its natural environment. A polypeptide preparation is substantially purified such that the polypeptide represents several embodiments at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 98%, of the total polypeptide content of the preparation. The same applies for polynucleotides. The polypeptides disclosed herein can be purified by any of the means known in the art (see, for example, *Guide to Protein Purification*, Deutscher (ed.), *Meth. Enzymol.* 185, Academic Press, San Diego, 1990; and Scopes, *Protein Purification: Principles and Practice*, Springer Verlag, New York, 1982).

Recombinant: A recombinant polynucleotide is one that has a sequence that is not naturally occurring or has a sequence that is made by an artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by chemical synthesis or, more commonly, by the artificial manipulation of isolated segments of nucleic acids, for example, by genetic engineering techniques. In one embodiment, a recombinant polynucleotide encodes a fusion protein.

Selectively hybridize: Hybridization under moderately or highly stringent conditions that excludes non-related nucleotide sequences.

In nucleic acid hybridization reactions, the conditions used to achieve a particular level of stringency will vary, depending on the nature of the nucleic acids being hybridized. For example, the length, degree of complementarity, nucleotide sequence composition (for example, GC v. AT content), and nucleic acid type (for example, RNA v. DNA) of the hybridizing regions of the nucleic acids can be considered in selecting hybridization conditions. An additional consideration is whether one of the nucleic acids is immobilized, for example, on a filter.

A specific, non-limiting example of progressively higher stringency conditions is as follows: 2 x SSC/0.1% SDS at about room temperature (hybridization conditions); 0.2 x SSC/0.1% SDS at about room temperature (low stringency conditions); 0.2 x SSC/0.1% SDS at about 42°C (moderate stringency conditions); and 0.1 x SSC at about 68°C (high stringency conditions). One of skill in the art can readily determine variations on these conditions (for example, *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, ed. Sambrook *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). The hydridization conditions can be carried out over 2 to 16 hours. Washing can be carried out using only one of the above conditions, for example, high stringency

conditions, or each of the conditions can be used, for example, for 10-15 minutes each, in the order listed above, repeating any or all of the steps listed. However, as mentioned above, optimal conditions will vary, depending on the particular hybridization reaction involved, and can be determined empirically.

Sequence identity: The similarity between amino acid sequences is expressed in terms of the percentage identity between the sequences. The higher the percentage, the more similar the two sequences are. Homologs or variants of a *Lu. longipalpis* polypeptide will possess a relatively significant high degree of sequence identity when aligned using standard methods.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman, *Adv. Appl. Math.* 2:482, 1981; Needleman and Wunsch, *J. Mol. Biol.* 48:443, 1970; Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988; Higgins and Sharp, *Gene* 73:237, 1988; Higgins and Sharp, *CABIOS* 5:151, 1989; Corpet *et al.*, *Nucleic Acids Research* 16:10881, 1988; and Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85:2444, 1988. Altschul *et al.*, *Nature Genet.* 6:119, 1994 presents a detailed consideration of sequence alignment methods and identity calculations.

The NCBI Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, *J. Mol. Biol.* 215:403, 1990) is available from several sources, including the National Center for Biotechnology Information (NCBI, Bethesda, MD) and on the Internet, for use in connection with the sequence analysis programs blastp, blastn, blastx, tblastn, and tblastx. A description of how to determine sequence identity using this program is available on the NCBI website on the internet.

Homologs and variants of a *Lu. longipalpis* polypeptide are typically characterized by possession of at least 75%, for example at least 80%, sequence identity counted over the full length alignment with the amino acid sequence of the *Lu. longipalpis* polypeptide using the NCBI Blast 2.0, gapped blastp set to default parameters. The comparison between the sequences is made over the full length alignment with the amino acid sequence given in this present disclosure, employing the Blast 2 sequences function using the default BLOSUM62 matrix set to default parameters, (gap existence cost of 11, and a per residue gap cost of 1).

When aligning short peptides (fewer than around 30 amino acids), the alignment should be performed using the Blast 2 sequences function, employing the PAM30 matrix set to default parameters (open gap 9, extension gap 1 penalties). Proteins with even greater similarity to the reference sequences will show increasing percentage identities when assessed by this method, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% sequence identity. When less than the entire sequence is being compared for sequence identity, homologues and, variants will typically possess at least 80% sequence identity over short windows of 10-20 amino acids, and may possess sequence identities of at least 85% or at least 90% or 95% depending on their similarity to the reference sequence. Methods for determining sequence identity over such short windows are available at the NCBI website on the internet. One of skill in the art will appreciate that these sequence identity ranges are provided for guidance only; it is entirely possible that strongly significant homologues could be obtained that fall outside of the ranges provided.

Specific binding agent: An agent that binds substantially only to a defined target. Thus a *Lu. longipalpis* specific binding agent is an agent that binds substantially to a *Lu. longipalpis* polypeptide.

In one embodiment, the specific binding agent is a monoclonal or polyclonal antibody that specifically binds the *Lu. longipalpis* polypeptide.

Subject: Living multi-cellular vertebrate organisms, a category that includes both human veterinary subjects, including human and non-human mammals. In one embodiment, the subject is a member of the canine family, such as a dog. In another embodiment, the subject is a human.

T Cell: A white blood cell critical to the immune response. T cells include, but are not limited to, $CD4^+$ T cells and $CD8^+$ T cells. A $CD4^+$ T lymphocyte is an immune cell that carries a marker on its surface known as "cluster of differentiation 4" (CD4). These cells, also known as helper T cells, help orchestrate the immune response, including antibody responses as well as killer T cell responses. $CD8^+$ T cells carry the "cluster of differentiation 8" (CD8) marker. In one embodiment, a CD8 T cells is a cytotoxic T lymphocytes. In another embodiment, a CD8 cell is a suppressor T cell.

Therapeutically active polypeptide: An agent, such as a *Lu. longipalpis* polypeptide, that causes induction of an immune response, as measured by clinical response (for example, increase in a population of immune cells, production of antibody that specifically binds the *Lu. longipalpis* polypeptide, a measurable reduction in symptoms resulting from exposure to *Leishmania*, or protection from infection with *Leishmania*). Therapeutically active molecules can also be made from nucleic acids. Examples of a nucleic acid based therapeutically active molecule is a nucleic acid sequence that encodes a *Lu. longipalpis* polypeptide, wherein the nucleic acid sequence is operably linked to a control element such as a promoter. Therapeutically active agents can also include organic or other chemical compounds that mimic the effects of the *Lu. longipalpis* polypeptide.

The terms "therapeutically effective fragment of a *Lu. longipalpis* polypeptide" includes any fragment of the *Lu. longipalpis* polypeptide, or variant of the *Lu. longipalpis* polypeptide, or fusion protein including a *Lu. longipalpis* polypeptide, that retains a function of the *Lu. longipalpis* polypeptide (such as immunogenicity), or retains the ability to reduce the symptoms from exposure to *Leishmania,* or to protect from infection with *Leishmania.*

Thus, in one embodiment, a therapeutically effective amount of a fragment of *Lu. longipalpis* polypeptide is an amount used to generate an immune response to the polypeptide. In another embodiment, a therapeutically effective amount of a fragment of a *Lu. longipalpis* polypeptide is an amount of use to prevent or treat a *Leishmania* infection in a subject. Treatment refers to a therapeutic intervention that confers resistance to infection with *Leishamania*, or a reduction in the symptoms associated with exposure to *Leishamania*. Specific, non-limiting examples of a polypeptide fragment are the N-terminal half or the C-terminal half of one of the *P. Lu. longipalpis* polypeptide disclosed herein. It should be noted that fusion proteins are included, such as a fusion with six histidine residues, a c-myc tag, or any other polypeptide tag. Such fusions are known to one of skill in the art, and are often used in protein purification.

Transduced: A transduced cell is a cell into which has been introduced a nucleic acid molecule by molecular biology techniques. As used herein, the term transduction encompasses all

techniques by which a nucleic acid molecule might be introduced into such a cell, including transfection with viral vectors, transformation with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration.

Vector: A nucleic acid molecule as introduced into a host cell, thereby producing a transduced host cell. A vector may include nucleic acid sequences that permit it to replicate in a host cell, such as an origin of replication. A vector may also include one or more selectable marker genes and other genetic elements known in the art.

Vaccine: Composition that when administered to a subject, induces a decrease of the severity of the symptoms of a disorder or disease. In one embodiment, a vaccine decreases the severity of the symptoms of leishmaniasis and/or decreases the parasitic load.

Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. "Comprise" means "include," and a composition that comprises a polypeptide includes that polypeptide. It is further to be understood that all base sizes or amino acid sizes, and all molecular weight or molecular mass values, given for polynucleotides or polypeptides are approximate, and are provided for description. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including explanations of terms, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

Lu. longipalpis Polynucleotides and Polypeptides

Salivary polypeptides from sand fly species *Lu. longipalpis,* are disclosed herein.

LJL34 (SEQ ID NO: 1)
MLQIKHLLIFVGLLVVVNAQSNYCKQESCSSGGVERPHIGCKNSGDFSETCSGDAEIVKMDK
KKQNLLVKMHNRLRDRFARGAVPGFAPAAKMPMLKWNDELAKLAEYNVRTCKFAHDKC
RAIDVCPYAGQNLAQMMSYPTHRDLNYVLKNLTREWFWEYRWAKQSQLDNYVGGPGKD
NKQIGHFTAFVHEKTDKVGCAIARFTNEHNFKETLLACNYCYTNMMKERIYTQGKPCSQCQ
SKKCGPVYKNLCDPSEKVDPTPDVLKQWKHGK

LJL18 (SEQ ID NO:3)
MLLRSLFVLFLIFLTFCNAEEELIERKLTGKTIYISTIKLPWFQALNHCVKNGYTMVSIKTFEE
NKELLKELKRVIRTEDTQVWIGGLKHHQFANFRWVSDGSHVATASGYTNWAPGEPADSFY
YDQFCMAMLFRKDGAPWDDLNCWVKNLFVCEKRDD

LJS193 (SEQ ID NO:5)
MKLLQIIFSLFLVFFPTSNGALTGNESAANAAPLPVVLWHGMGDSCCFPFSLGSIKKLIEQQIP
GIHVVSLKIGKSLIEDYESGFFVHPDKQIQEVCESLQNDLTLANGFNAIGFSQGSQFLRGLVQR
CSSIQVRNLISIGGQHQGVFGLPYCPSLSRKTCEYFRKLLNYAAYEKWVQKLLVQATYWHD
PLNEDAYRTGSTFLADINNERQINNDYINNIRKLNRFVMVKFLNDSMVQPIESSFFGFYAPGT
DTEVLPLKQSKIYLEDRLGLQSVPIDYLECGGDHLQFTKEWFIKFIIPYLKQ

LJS201 (SEQ ID NO: 7)
MRNFAVVSLAVAVLLFCAWPINAEDNEEVGKAREKRGLKDAMEHFKNGFKELTKDFKLPS
LPSLPGFGKKPESGSSEDSGDKTEDTSGSKDDQSKDNTVEES

LJL13 (SEQ ID NO: 9)
MNFLLKIFSLLCLCGLGYSWQDVRNADQTLWAYRSCQKNPEDKDHVPQWRKFELPDDEKT
HCYVKCVWTRLGAYNENENVFKIDVITKQFNERGLEVPAGLDQELGGSTDGTCKAVYDKS
MKFFKSHFMDFRNAYYATYDGSDEWFSKNPDVKPKGTKVSEYCKNKDDGDCKHSCSMYY
YRLIDEDNLVIPFSNLPDYPEDKLEECRNEAKSANECKSSVIYQCLENADKSALDASLNIL
DEFSGRY

LJL23 (SEQ ID NO: 11)
MFLKWVVCAFATVFLVGVSQAAPPGVEWYHFGLIADMDKKSIASDKTTFNSVLKIDELRHN
TKTDQYIYVRSRVKKPVSTRYGFKGRGAELSEIVVFNNKLYTVDDKSGITFRITKDGKLFPW
VILADADGQRPDGFKGEWATIKDDTIYVGSTGMLKFTSSLWVKKITKDGVVTSHDWTDKY
RKILKALNMPNGFVWHEAVTWSPFRKQWVFMPRKCSRHPFSQELEERTGCNKIVTADENFN
DIQVIHIQDQPYNLASGFSSFRFIPGTKNERLLALRTVEQEDQVKTWAVVMDMKGTVLMYE
KELYDEKFEGLAFFGGIKKN

LJM10 (SEQ ID NO: 13)
MALKFLPVLLLSCFAMSTALQVTEKELSDGKKIFISKVELNWFEALDFCIHRGLTLLSIKSAK
ENVDVTKAIRAELNFDSKKLAHVWTGGIRHSQDKYFRWINDGTKVVKRVYTNWFTGEPNN
GYWKDEFCLEIYYKTEEGKWNDDKCHVKHHFVCQEKK

LJL143 (SEQ ID NO: 15)
MNSINFLSIVGLISFGFIVAVKCDGDEYFIGKYKEKDETLFFASYGLKRDPCQIVLGYKCSNN
QTHFVLNFKTNKKSCISAIKLTSYPKINQNSDLTKNLYCQTGGIGTDNCKLVFKKRKRQIAAN
IEIYGIPAKKCSFKDRYIGADPLHVDSYGLPYQFDQEHGWNVERYNIFKDTRFSTEVFYHKN
GLFNTQITYLAEEDSFSEAREITAKDIKKKFSIILPNEEYKRISFLDVYWFQETMRKKPKYPYIH
YNGECSNENKTCELVFDTDELMTYALVKVFTNPESDGSRLKEEDLGRG

LJS142 (SEQ ID NO: 17)
MAFSNTLFVLFVSFLTFCGADQTLIEKELTGRTVYISKIKLNWNDAFDYCIRNGLTFAKIKSA
EENTELSEKLKTVIRTEEFQVWIGGIEHHQDSSFRWVSDSQPITNKLGYKYTNWNTGEPTNY
QNNEYCLEILFRKEDGKWNDFPCSARHHFVCEKRTK

LJL17 (SEQ ID NO: 19)
MQNFLLVSLALAALMLCAEAKPYDFPLYQDLIQGVIQRESQAEREKRSPNEDYEKQFGDIVD
QIKEISFNVMKMPHFGSSDDNRDDGEYVDHHYGDEDDRDYDHY

LJM06 (SEQ ID NO: 21)
MKFYIFGVFLVSFLALCNAEDYDKVKLTGRTVYISRSKAPWFTALDNCNR
RFTFAMIKSQKENEELTNALLSVIKSDEENVWIGGLRHDLDDYFRWISFGTALSKTSYTNWA
PKEPTGRPHRTQNDEFCMQMSFKDGGKWSDNTCWRKRLYVCEKRD

LJM17 (SEQ ID NO: 23)
MRFFFVFLAIVLFQGIHGAYVEIGYSLRNITFDGLDTDDYNPKFNIPTGLAVDPEGYRLFIAIPR
RKPKVPYTVAELNMVMNPGFPVERAPSFEKFKKFNGEGKKDLVNVYQPVIDDCRRLWVLDI
GKVEYTGGDADQYPKGKPTLIAYDLKKDHTPEIHRFEIPDDLYSSQVEFGGFAVDVVNTKG
DCTESFVYLTNFKDNSLIVYDETQKKAWKFTDKTFEADKESTFSYSGEEQMKYKVGLFGIAL
GDRDEMGHRPACYIAGSSTKVYSVNTKELKTENGQLNPQLHGDRGKYTDAIALAYDPEHK
VLYFAESDSRQVSCWNVNMELKPDNTDVIFSSARFTFGTDILVDSKGMLWIMANGHPPVED
QEKIWKMRFVNRKIRIMKVDTERVFKYSRCNPNYKPPK
EIEV

LJL04 (SEQ ID NO: 25)
MIKEVFSLALLVALAQCANEIPINRQGKDYPVPIIDPNKSSSDDYFDDRFYPDIDDEGIAEAPK
DNRGKSRGGGAAGAREGRLGTNGAKPGQGGTRPGQGGTRPGQGGTRPGQGGTRPGQGGT
RPGQGRTKPAQGTTRPAQGTRNPGSVGTKEAQDASKQGQGKRRPGQVGGKRPGQANAPNA
GTRKQQKGSRGVGRPDLSRYKDAPAKFVFKSPDFSGEGKTPTVNYFRTKKKEHIVTRGSPN
DEFVLEILDGDPTGLGLKSETIGKDTRLVLENPNGNSIVARVKIYKNGYSG

LJM114 (SEQ ID NO: 27)
MNSVNTLILTLLFAIFLLVKRSQAFLPSDPSICVKNLVLDTGRTCEESEYFPDIKNVKNGKRVY
IVCTDSDAVDYKFYICFDMNRLSGPPYPEEEILRESTVTYAQIYELMTTETTETKKPKKKPKN
SKTDDPPAIRPGFSFRNSISV

LJM111 (SEQ ID NO: 29)
MKLFFFLYTFGLVQTIFGVEIKQGFKWNKILYEGDTSENFNPDNNILTAFAYDPESQKLFLTV
PRKYPETMYTLAEVDTEKNSFESGDTSPLLGKFSGHETGKELTSVYQPVIDECHRLWVVDVG

SVERNSDGTEGQPEHNPTLVAYDLKEANYPEVIRYTFPDNSIEKPTFLGGFAVDVVKPDECSE
TFVYITNFLTNALIVYDHKNKDSWTVQDSTFGPDKKSKFDHDGQQYEYEAGIFGITLGERDN
EGNRQAYYLVASSTKLHSINTKELKQKGSKVNANYLGDRGESTDAIGLVYDPKTKTIFFVES
NSKRVSCWNTQETLNKDKIDVIYHNADFSFGTDISIDSQDNLWFLANGLPPLENSDKFVFTKP
RYQIFKVNIQEAIAGTKCEKNL

LJM78 (SEQ ID NO: 31)
MTFLIILGAFLLVQIITASALGLPEQFKGLEDLPKKPLAETYYHEGLNDGKTDEMVDIFKSLSD
EFKFSDENLDVGEEKNYKKRDITQNSVARNFLSNVKGIPSMPSLPSMPSMPSIPSLWSSQTQA
APNTALALPESDYSLLDMPNIVKNFLKETRDLYNDVGAFLKAITEALTNRSSSSQLLSSPMVS
TNKTKEFIRNEIQKVRKVRNFVQETLQKIRDISAAIAKKVKSSECLSNLTDIKGLVSDGINCLK
EKFNDGKRIILQLYNNLLKGLKIPNDLMVELKKCDTNQNNTLGRIICYFLTPLQLEKEQILLPV
EFIKRILELTHYFSTMKEDLINCGITTIASIT

LJS238 (SEQ ID NO:33)
MLKIVLFLSVLAVLVICVAAMPGSNVPWHISREELEKLREARKNHKALEKAIDELIDKYL

LJS169 (SEQ ID NO:35)
MKFSCPVFVAIFLLCGFYRVEGSSQCEEDLKEEAEAFFKDCNEAKANPGEYENLTKEEMFEE
LKEYGVADTDMETVYKLVEECWNELTTTDCKRFLEEAECFKKKNICKYFPDEVKLKKK

LJL11 (SEQ ID NO: 37)
MLFFLNFFVLVFSIELALLTASAAAEDGSYEIIILHTNDMHARFDQTNAGSNKCQEKDKIASK
CYGGFARVSTMVKKFREENGSSVLFLNAGDTYTGTPWFTLYKETIATEMMNILRPDAASLG
NHEFDKGVEGLVPFLNGVTFPILTANLDTSQEPTMTNAKNLKRSMIFTVSGHRVGVIGYLTP
DTKFLSDVGKVNFIPEVEAINTEAQRLKKEENAEIIIVVGHSGLIKDREIAEKCPLVDIIVGGHS
HTFLYTGSQPDREVPVDVYPVVVTQSSGKKVPIVQAYCFTKYLGYFKVTINGKGNVVGWTG
QPILLNNNIPQDQEVLTALEKYRERVENYGNRVIGVSRVILNGGHTECRFHECNMGNLITDA
FVYANVISTPMSTNAWTDASVVLYQSGGIRAPIDPRTAAGSITRLELDNVLPFGNALYVVKV
PGNVLRKALEHSVHRYSNTSGWGEFPQVSGLKIRFNVNEEIGKRVKSVKVLCSNCSQPEYQP
LRNKKTYNVIMDSFMKDGGDGYSMFKPLKIIKTLPLGDIETVEAYIEKMGPIFPAVEGRITV
LGGLQKSDEDWH

LJL08 (SEQ ID NO: 39)
MKQILLISLVVILAVLAFNVAEGCDATCQFRKAIEDCKKKADNSDVLQTSVQTTATFTSMDT
SQLPGNNVFKACMKEKAKEFRAGK

LJS105 (SEQ ID NO: 41)
MNVLFVSFTLTILLLCVKARPEDFVALQDQANFQKCLEQYPEPNQSGEVLACLKKREGAKD
FREKRSLDDIEGTFQESGN
LWGA

LJL09 (SEQ ID NO: 43)
MKITVILFTGFTIALVSSAVLKKNGETIEEEEVRAEQRLREINEELDRRKNINTVAAWAYASNI
TEVNLKNMNDVSVETAKYYKELASELKGFNAKEYKSEDLKRQIKKLSKLGYSALPSEKYKE
LLEAITWMESNYAKVKVCSYKDPKKCDLALEPEITEILIKSRDPEELKYYWKQWYDKAGTP
TRESFNKYVQLNREAAKLDGFYSGAESWLDEYEDETFEKQLEDIFAQIRPLYEQLHAYVRFK
LREKYGNDVVSEKGPIPMHLLGNMWGQTWSEVAPILVPYPEKKLLDVTDEMVKQGYTPIS
MFEKGDEFFQSLNMTKLPKTFWEYSILEKPQDGRELICHASAWDFYTKDDVRKQCTRVTMD
QFFTAHHELGHIQYYLQYQHLPSVYREGANPGFHEAVGDVLSLSVSSPKHLEKVGLLKDFKF
DEESQINQLLNLALDKMAFLPFAYTIDKYRWGVFRGEISPSEYNCKFWEMRSYYGGIEPPIAR
SESDFDPPAKYHISSDVEYLRYLVSFIIQFQFHQAVCQKTGQFVPNDPEKTLLNCDIYQSAEA
GNAFKEMLKLGSSKPWPDAMEILTGQRKMDASALIEYFRPLSEWLQKKNKELGAYVGWDK
STKCVKNVS

LJL38 (SEQ ID NO: 45)
MKTFALIFLALAVFVLCIDGAPTFVNLLDDVQEEVEVNTYEP

LJM04 (SEQ ID NO:47)
MNHLCFIIIALFFLVQQSLAEHPEEKCIRELARTDENCILHCTYSYYGFVDKNFRIAKKHVQKF
KKILVTFGAVPKKEKK
KLLEHIEACADSANADQPQTKDEKCTKINKYYRCVVDGKILPWNSYADAIIKFDKTLNV

LJM26 (SEQ ID NO: 49)
MKIIFLAAFLLADGIWAAEEPSVEIVTPQSVRRHATPKAQDARVGSESATTAPRPSESMDYW
ENDDFVPFEGPFKDIGEFDWNLSKIVFEENKGNAILSPLSVKLLMSLLFEASASGTLTQHQLR
QATPTIVTHYQSREFYKNIFDGLKKKSNDYTVHFGTRIYVDQFVTPRQRYAAILEKHYLTDL
KVEDFSKAKETTQAINSWVSNITNEHIKDLVKEEDVQNSVMLMLNAVYFRGLWRKPFNRTL
PLPFHVSADESKTTDFMLTDGLYYFYEAKELDAKILRIPYKGKQYAMTVILPNSKSGIDSFVR
QINTVLLHRIKWLMDEVECRVILPKFHFDMTNELKESLVKLGISQIFTSEASLPSLARGQGVQ
NRLQVSNVIQKAGIIVDEKGSTAYAASEVSLVNKFGDDEFVMFNANHPFLFTIEDETTGAILF
TGKVVDPTQ

LJS03 (SEQ ID NO: 51)
MRFLLLAFSVALVLSPTFAKPGLWDIVTGINDMVKNTANALKNRLTTSVTLFTNTITEAIKNA
NSSVSELLQQVNETLTDIINGVGQVQSAFVNSAGNVVVQIVDAAGNVLEVVVDEAGNIVEV
AGTALETIIPLPGVVIQKIIDALQGNAGTTSDSASSTVPQQS

LJS192 (SEQ ID NO: 53)
MVKYSCLVLVAIFLLAGPYGVVGSCENDLTEAAKYLQDECNAGEIADEFLPFSEEEVGEALS
DKPENVQEVTNIVRGCFEAEQAKEHGKCERFSALSQCYIEKNLCQFF

LJM19 (SEQ ID NO: 55)
MKFFYLIFSAIFFLADPALVKCSEDCENIFHDNAYLLKLDCEAGRVDPVEYDDISDEEIYEITV
DVGVSSEDQEKVAKIIRECIAQVSTQDCTKFSEIYDCYMKKKICNYYPENM

LJL138 (SEQ ID NO: 57)
MHLQLNLCAILLSVLNGIQGAPKSINSKSCAISFPENVTAKKEPVYLKPSNDGSLSTPLQPSGP
FVSLKIGESLAIFCPGDGKDVETITCNTNFDLASYSCNKSTSTDTIETEEVCGGSGKVYKVGFP
LPSGNFHSIYQTCFDKKNLTPLYSIHILNGQA
VGYHLKHTRGSFRTNGIYGKVNIDKLYKTQIEKFNKLFGPKQTFFRRPLNFLSRGHLSPEVDF
TFRREQHATEMYINTAPQYQSINQGNWLRVENHVRDLAKVLQKDITVVTGILGILRLKSKKI
EKEIYLGDDVIAVPAMFWKAVFDPQKQEAIVFVSSNNPHVKTFNPNCKDVCAQAGFGNDNL
EYFSNYSIGLTICCKLEEFVKRNKIILPKEVNNKNYTKKLLKFPKTRNKEGDKKVVRKRAKG
A

LJL15 (SEQ ID NO: 59)
MNLHLAIILFVSYFTLITATDLIEKELSDCKKIFISKAELTWFQALDFCTEQNLTLLSIKSAREN
DEVTKAVRAEVHLPDTKKSHIWLGGIRYDQDKDFRWISDGTTVTKTVYINWYQGEPNGGR
YQKEFCMELYFKTPAGQWNDDICTAKHHFICQEKK

LJL91 (SEQ ID NO: 61)
MNLPLAIILFVSYFTLITAADLTEKELSDGKKIFISKAELSWFDALDACTEKDLTLLTIKSAREN
EEVTKAVRAEVHLPDTKKSHIWLGGIRYDQDKDFRWISDGTTVTKTVYINWYQGEPNGGRY
QKEFCMELYFKTPAGQWNDDICTAKHHFICQEKK

LJM11 (SEQ ID NO: 63)
MKVFFSIFTLVLFQGTLGADTQGYKWKQLLYNNVTPGSYNPDNMISTAFAYDAEGEKLFLA
VPRKLPRVPYTLAEVDTKNSLGVKGKHSPLLNKFSGHKTGKELTSIYQPVIDDCRRLWVVDI
GSVEYRSRGAKDYPSHRPAIVAYDLKQPNYPEVVRYYFPTRLVEKPTYFGGFAVDVANPKG
DCSETFVYITNFLRGALFIYDHKKQDSWNVTHPTFKAERPTKFDYGGKEYEFKAGIFGITLGD
RDSEGNRPAYYLAGSAIKVYSVNTKELKQKGGKLNPELLGNRGKYNDAIALAYDPKTKVIF

FAEANTKQVSCWNTQKMPLRMKNTDVVYTSSRFVFGTDISVDSKGGLWFMSNGFPPIRKSE
KFKYDFPRYRLMRIMDTQEAIAGTACDMNA

LJS138 (SEQ ID NO: 65)
MQSKILSFVLFTLSLGYVLGETCSNAKVKGATSYSTTDATIVSQIAFVTEFSLECSNPGSEKISL
FAEVDGKITPVAMIGDTTYQVSWNEEVNKARSGDYSVKLYDEEGYGAVRKAQRSGEENKV
KPLATVVVRHPGTYTGPWFNSEILAAGLIAVVAYFAFSTRSKILS

LJL124 (SEQ ID NO: 67)
MVSILLISLILNLLVFYAKARPLEDISSDLSPDYYITEGYDGVKEKREIELVPVTFGIFNIHTTPA
PRITFEW

LJL35 (SEQ ID NO: 69)
MKLFCLIFVVFVALEVCIETVKAMEATEEISVKLQDDANEPDDSLDLDEGLPDAFDEDYNNQ
AEYKPNPRGDYRRR

In one embodiment, a polypeptide including SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 is disclosed herein. Homologous polypeptides having an amino acid sequence at least 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 are disclosed herein. Fusion proteins including SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 are also disclosed herein.

Fragments and variants of the *Lu. longipalpis* polypeptides identified above are disclosed herein and can readily be prepared by one of skill in the art using molecular techniques. In one embodiment, a fragment of a *Lu. longipalpis* polypeptide includes at least 8, 10, 15, or 20 consecutive amino acids of a *Lu. longipalpis* polypeptide. In another embodiment, a fragment of a *Lu.*

longipalpis polypeptide includes a specific antigenic epitope found on a full-length *Lu. longipalpis* polypeptide.

In one embodiment, a fragment is at least 19 amino acids, at least 23 amino acids, at least 25 amino acids, or at least 30 amino acids in length from any polypeptide (including polypeptides as given in SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 conservative variants thereof, and homologues thereof), or any fragment that retains at least an epitope.

Fusion proteins including a *Lu. longipalpis* polypeptide can also be produced using methods known to one of skill in the art. In one embodiment, a fusion protein includes an amino acid sequence set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or a conservative variants thereof, and a marker polypeptide. Marker polypeptides include, but are not limited to, polypeptide tags, such as a polypeptide to aid in protein purification (for example, six histidine residues or c-myc polypeptide), or an enzymatic marker (for example, alkaline phosphatase), or a fluorescent maker (for example, green fluorescent protein).

One skilled in the art, given the disclosure herein, can purify a *Lu. longipalpis* polypeptide using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the *Lu. longipalpis* polypeptide can also be determined by amino-terminal amino acid sequence analysis.

Minor modifications of the *Lu. longipalpis* polypeptide primary amino acid sequences may result in peptides which have substantially equivalent activity as compared to the unmodified counterpart polypeptide described herein. Such modifications may be deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein.

Polynucleotides encoding salivary polypeptides from *Lu. longipalpis* sand fly are disclosed herein, such as polynucleotides encoding SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67.

Specific, non-limiting examples of *Lu. longipalpis* nucleic acid sequences include SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:16, SEQ ID NO:18, SEQ ID

NO:20, SEQ ID NO:22, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:42, SEQ ID NO:44, SEQ ID NO:46, or SEQ ID NO:48, SEQ ID NO:50, SEQ ID NO:52, SEQ ID NO:54, SEQ ID NO:56, SEQ ID NO:58, SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:64, SEQ ID NO:66, SEQ ID NO:68, and degenerate variants thereof. These polynucleotides include DNA, cDNA, and RNA sequences that encode a *Lu. longipalpis* polypeptide. It is understood that all polynucleotides encoding a *Lu. longipalpis* polypeptide are also included herein, as long as they encode a polypeptide with the recognized activity, such as the binding to an antibody that recognizes the polypeptide, the induction of an immune response to the polypeptide, or an effect on survival of *Leishmania* when administered to a subject having leishmaniasis or who undergoes a decrease in a sign or a symptom of *Leishmania* infection.

The polynucleotides of the disclosure include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the disclosure as long as the amino acid sequence of the *Lu. longipalpis* polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specific, non-limiting examples of a polynucleotide encoding a *P. ariasi* polypeptide are set forth below:

LJL34 (SEQ ID NO:2)

AGTTGTGGAGCTTTTGGTCATTTTACGTGATGTTGCAAATTAAACATCTTCTGATTTTTGT
GGGATTGCTCGTGGTTGTTAATGCACAGAGCAATTACTGCAAACAGGAATCGTGCTCAT
CGGGAGGTGTTGAGAGACCCCATATTGGGTGCAAAAACTCTGGAGATTTTTCCGAAACT
TGCTCCGGAGATGCAGAAATTGTTAAGATGGACAAGAAGAAGCAGAACCTCCTTGTGAA
AATGCACAATCGCCTGAGAGATAGATTTGCTCGTGGTGCAGTGCCAGGTTTTGCACCAG
CTGCGAAAATGCCAATGCTTAAATGGAACGATGAACTGGCCAAATTGGCAGAGTACAAC
GTGAGAACGTGCAAATTTGCCCACGATAAATGCCGCGCAATTGATGTCTGCCCCTATGCT
GGACAGAATCTAGCTCAAATGATGTCCTATCCTACCCATCGAGATCTAAACTATGTTCTT
AAGAATCTCACAAGGGAATGGTTCTGGGAGTACAGATGGGCTAAGCAATCTCAGCTTGA
TAATTACGTGGGTGGTCCTGGGAAAGACAACAAACAAATTGGACATTTCACAGCTTTTG
TGCATGAGAAAACAGACAAAGTTGGATGCGCTATAGCTCGATTTACAAATGAGCACAAT
TTTAAGGAGACCCTCCTAGCTTGCAACTACTGCTACACGAATATGATGAAGGAGAGGAT
CTACACGCAGGGAAAACCTTGTTCACAGTGTCAGAGCAAAAAGTGTGGGCCAGTCTACA
AGAACCTGTGTGATCCTTCGGAGAAGGTTGATCCAACTCCTGATGTCCTTAAGCAATGG
AAGCATGGAAAATGATTATTAAGCTCACTTCAAATGTTTCCAATCCAAAAAAAAAAAAA
AAAAAAAAAAAAAAAA

LJL18 (SEQ ID NO:4)

TTTTGAGAAAAACATTTCCTTGTGAGTTAAATAGTTGGTAAATTAAATCAAGAGAATGTT
GCTTCGTTCCTTGTTTGTTCTTTTTCTAATTTTCTTAACATTCTGCAACGCTGAGGAAGAA

CTTATTGAGAGAAAGTTAACAGGAAAAACGATCTATATCTCAACAATAAAGCTTCCGTG
GTTCCAAGCTCTTAATCATTGTGTTAAAAATGGCTACACAATGGTGTCAATTAAGACATT
TGAAGAGAATAAAGAACTCCTTAAAGAACTCAAAAGGGTGATTAGGACAGAAGATACA
CAAGTTTGGATTGGAGGCCTCAAACATCATCAATTTGCAAACTTTCGTTGGGTAAGCGAT
GGAAGCCACGTAGCAACAGCTTCAGGGTACACCAATTGGGCCCCAGGGGAGCCAGCTG
ATTCCTTCTATTACGATCAATTTTGCATGGCGATGTTGTTCAGAAAAGACGGCGCTCCGT
GGGATGATTTGAATTGTTGGGTTAAGAATCTTTTTGTTTGTGAGAAACGAGATGATTGAG
AGGCTATTTTTGTTATCTCACCGTTTTGTTGAATAAAAAAGAAGAAGAAAGACAAAAAA
AAAAAAAAAAAAAAAAAAAAAA

LJS193 (SEQ ID NO:6)

TACTTCGTACTCTCAGAATTTCTTACAAGTTCCTTTTTCTCTTAACTTTTAAAGTTTTATTT
AACAAAATTGCTCCATTTTTTCGTTTTCTGAATATTCTGTTGAAATTTTGATTAATCTATT
TTATGTGCAGTTTTTACTAAAAATCCCTTATCAGCAACCCGGTGTCTACAGTTTTGTCAC
GCTCAGTAGCATCTTCAAGGTGGTAAGAAAAAATGAAACTCCTGCAAATCATCTTCTCTC
TCTTCCTGGTCTTTTTCCCGACCTCAAATGGGGCCCTGACCGGAAATGAAAGTGCAGCAA
ATGCAGCTCCCTTGCCTGTCGTCCTGTGGCACGGGATGGGCGATTCTTGCTGCTTTCCCTT
CAGTTTGGGAAGCATAAAAAAAATTAATTGAACAACAAATTCCTGGGATTCATGTTGTTA
GCCTGAAAATTGGAAAGTCTCTCATTGAGGACTATGAAAGTGGATTTTTTGTTCATCCAG
ACAAGCAAATTCAGGAAGTTTGTGAGTCACTTCAGAACGATCTAACACTCGCAAATGGA
TTCAATGCAATTGGATTTTCTCAGGGTAGTCAGTTCCTGCGAGGTCTTGTGCAACGATGT
TCTTCTATACAAGTAAGGAATCTCATTTCCATTGGAGGACAGCATCAAGGGGTTTTTGGT
CTGCCCTATTGTCCTTCGTTGAGCAGAAAGACTTGCGAATACTTTAGAAAGCTCCTGAAT
TATGCAGCTTATGAAAAATGGGTACAGAAACTCCTAGTTCAAGCCACCTACTGGCATGA
TCCTCTAAATGAGGATGCATATCGGACTGGAAGCACTTTCCTTGCTGATATAAATAATGA
GAGACAAATCAATAATGACTATATTAATAATATTCGGAAGCTAAATCGTTTTGTGATGGT
AAAGTTCCTCAACGACAGCATGGTTCAGCCAATTGAATCTAGTTTCTTTGGATTCTACGC
TCCAGGAACTGATACAGAAGTTCTCCCATTAAAACAAAGCAAGATTTATTTGGAAGATC
GTTTGGGACTTCAATCAGTACCGATAGATTATCTAGAATGCGGAGGAGATCATTTGCAA
TTTACAAAAGAATGGTTCATAAAGTTTATCATACCCTATCTGAAGCAATAAGAGCTGCA
ATGTAATTGATTAAAAAATGTTAACCATTTCAGGATGATTGGGTGACCCCTTAAAAATAT
AAATGAAAAAATATACAAAAGAAATAAATTTTTATATTGATCCCACAAAAAAAAAAAA
AAAAAAAAAAAAAAAAA

LJS201 (SEQ ID NO: 8)

GGATCGGCCATTATGGCCGGGGCAGTTAATCGCCACAATTTAATAAAATGAGGAACTTT
GCTGTAGTCAGTTTAGCCGTTGCTGTCCTGCTCTTCTGTGCATGGCCTATAAATGCGGAA
GATAATGAAGAAGTTGGAAAGGCGAGAGAAAAAAGAGGCTTAAAAGACGCAATGGAA
CACTTCAAAAATGGATTTAAGGAGCTGACAAAGGACTTTAAACTTCCAAGCCTTCCAAG

TCTTCCTGGATTTGGTAAAAAGCCTGAATCTGGAAGTTCTGAAGATTCTGGAGATAAAA
CTGAGGATACCAGTGGATCTAAGGACGACCAATCAAAGGATAATACGGTCGAAGAATCT
TAAGAAAGGCGCAAATAGCTATTTTCAAAGTGGCGAATGTTTCTTTCTTTATCTGAAATA
AATATTTTTAAACCTTTCGAAACCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL13 (SEQ ID NO:10)

ACTTAAAGATTTTTGTTTAAGCAAAATGAACTTCTTGTTGAAAATTTTCTCTTTGCTCTGT
CTCTGTGGACTGGGGTATTCATGGCAGGATGTGAGAAATGCCGATCAAACCCTCTGGGC
GTATAGATCGTGCCAAAAGAATCCTGAAGATAAGGATCACGTACCTCAATGGAGGAAGT
TCGAATTACCCGACGATGAAAAGACTCATTGCTACGTCAAGTGCGTATGGACGCGTTTG
GGAGCTTACAATGAAAATGAAAATGTTTTCAAAATTGATGTCATTACTAAGCAATTTAAT
GAACGTGGCCTAGAAGTTCCGGCTGGACTTGATCAAGAATTGGGTGGTTCTACAGATGG
AACTTGCAAAGCAGTTTACGATAAATCCATGAAGTTCTTCAAATCTCATTTTATGGACTT
TAGGAATGCTTACTACGCAACTTATGACGGTTCTGATGAATGGTTTAGCAAGAACCCTG
ATGTAAAACCGAAAGGAACAAAAGTTTCCGAATACTGCAAAAATAAAGATGATGGAGA
TTGCAAACATTCCTGCAGTATGTACTACTACCGCTTAATCGATGAAGACAACTTAGTTAT
TCCGTTCAGCAACTTACCTGACTATCCCGAAGATAAGCTCGAGGAATGCAGGAATGAAG
CCAAGTCCGCAAATGAGTGCAAATCATCTGTTATCTATCAGTGTTTGGAAAATGCGGAT
AAGTCAGCTTTAGACGCGTCTTTGAATATACTCGATGAGTTTTCTGGAAGATATTAAAAC
AAACTGGATAAAAAACTTAGGCCAACCTATGATTCGAACTTACGATTTTGAACTTGAAA
TTCATGTGCTTTAACCTATTGTCCCACTAGGAAGAAAAATCCATATTTGGTGATGTTAAA
CTATTTTTGAACCTCTTCAAAATAAACAATTTTCAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAA

LJL23 (SEQ ID NO:12)

AAAGAGAAGTAGTGAGAATGTTTCTTAAGTGGGTTGTTTGTGCTTTTGCGACTGTCTTCC
TTGTTGGGGTGAGTCAGGCAGCCCCACCGGGGGTTGAATGGTATCACTTTGGTCTGATTG
CTGATATGGACAAAAAATCCATCGCGAGTGACAAAACCACCTTTAACAGCGTCCTAAAG
ATCGATGAATTGCGCCACAACACAAAAACGGATCAATACATTTATGTGCGTAGTCGAGT
GAAGAAGCCCGTTTCCACGAGGTATGGGTTCAAAGGACGCGGTGCGGAATTGTCGGAAA
TTGTTGTCTTCAACAATAAACTTTACACAGTTGATGATAAATCTGGAATTACGTTCCGCA
TAACGAAAGACGGAAAACTCTTCCCGTGGGTTATTCTCGCAGATGCCGATGGACAGCGA
CCCGATGGCTTTAAGGGTGAATGGGCTACAATTAAGGATGATACAATCTATGTTGGATC
TACGGGGATGCTCAAGTTCACTTCATCCCTTTGGGTGAAGAAGATCACGAAAGATGGCG
TTGTTACGAGTCACGATTGGACTGATAAATACCGAAAGATTCTCAAAGCTCTAAACATG
CCAAATGGTTTTGTCTGGCATGAGGCTGTTACGTGGTCTCCATTCAGGAAGCAATGGGTC
TTCATGCCGAGAAAGTGCTCAAGGCATCCCTTCTCACAGGAACTCGAAGAACGCACAGG
GTGCAATAAAATAGTGACGGCAGATGAGAATTTCAACGACATTCAAGTTATTCACATTC

AAGATCAGCCATATAATTTAGCTTCTGGTTTCTCTTCCTTCCGCTTTATTCCTGGTACGAA
AAATGAAAGACTTCTCGCCTTGAGGACAGTAGAGCAGGAAGATCAGGTTAAAACTTGGG
CTGTGGTCATGGATATGAAAGGAACAGTTCTGATGTACGAAAAGGAACTTTATGACGAA
AAATTCGAAGGTTTAGCATTCTTTGGTGGTATTAAAAAGAATTAATTTGTTCCAGAAGCT
TTTAGATGAAATAATAAATTTTATTTCATTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AA

LJM10 (SEQ ID NO: 14)
CGCGGCCGCGTCGACCGACAGAAGGGGTAGTTTGTAGAGAACTTTGAGTTCTAAAGGAA
ATTCTCAAGAAGAAAATATTCAAAAGTAAAGAATGGCGTTGAAGTTTCTTCCGGTTCTCC
TTCTAAGCTGCTTCGCAATGAGCACGGCACTACAAGTTACTGAGAAGGAACTTTCTGAT
GGGAAAAAGATCTTCATCTCCAAAGTTGAGCTAAACTGGTTCGAAGCTCTTGATTTCTGT
ATCCATCGTGGTCTTACGTTGCTCTCAATTAAATCCGCCAAGGAAAATGTAGACGTAACA
AAAGCAATTCGGGCTGAATTGAATTTTGATTCAAAGAAATTGGCTCATGTGTGGACTGG
AGGTATTCGCCATAGTCAAGATAAGTATTTCCGTTGGATAAATGATGGAACTAAAGTTG
TTAAACGAGTCTACACCAATTGGTTCACTGGAGAACCAAATAATGGTTACTGGAAGGAT
GAATTTTGTCTGGAAATTTACTATAAAACCGAAGAAGGGAAGTGGAATGATGATAAATG
TCACGTGAAGCATCATTTTGTATGTCAAGAAAAGAAATAAATTGATTGATTTTGTTTGCT
GATTTGCAGTTCAGAATTGAAAAGCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL143 (SEQ ID NO: 16)
CTTCTTTGGATTTATTGAGTGATTAACAGGAAATTAGCTGAAGAAATGAATTCGATTAAT
TTCCTATCAATAGTTGGTTTAATCAGTTTTGGATTCATTGTTGCAGTAAAGTGTGATGGT
GATGAATATTTCATTGGAAAATACAAAGAAAAAGATGAGACACTGTTTTTTGCAAGCTA
CGGCCTAAAGAGGGATCCTTGCCAAATTGTCTTAGGCTACAAATGCTCAAACAATCAAA
CCCACTTTGTGCTTAATTTTAAAACCAATAAGAAATCCTGCATATCAGCAATTAAGCTGA
CTTCTTACCCAAAAATCAATCAAAACTCGGATTTAACTAAAAATCTCTACTGCCAAACTG
GAGGAATAGGAACAGATAACTGCAAACTTGTCTTCAAGAAACGTAAAAGACAAATAGC
AGCTAATATTGAAATCTACGGCATTCCAGCGAAGAAATGTTCCTTCAAGGATCGTTACAT
TGGAGCTGATCCACTCCACGTCGATTCCTATGGGCTTCCGTATCAGTTTGATCAGGAACA
TGGATGGAATGTGGAACGATATAACATTTTCAAAGACACAAGATTTTCCACAGAAGTTT
TCTACCACAAAAATGGTTTATTTAACACCCAAATAACTTATTTGGCTGAAGAAGATTCCT
TCTCTGAAGCTCGAGAGATTACTGCGAAGGATATTAAGAAGAAGTTTTCAATTATTTTGC
CCAATGAAGAGTATAAGAGGATTAGTTTCTTGGACGTTTATTGGTTCCAGGAGACTATGC
GAAAAAAGCCTAAATATCCCTACATTCACTACAATGGAGAATGCAGCAATGAGAATAAA
ACTTGTGAACTTGTCTTTGACACCGATGAACTAATGACCTACGCCCTTGTTAAAGTCTTT
ACTAATCCTGAGAGTGATGGATCTAGGCTCAAAGAAGAGGATTTGGGAAGAGGATAAA
TCTTCTTAATAAAAAAAAGTTCTGTAAGAAAATATTGTTCAATAAATTAAAAAAAAAAA
AAAAAAAAAA

LJS142 (SEQ ID NO: 18)

AATAGATCTTCAAAACGTCTAAGAATGGCTTTCAGCAACACTTTATTTGTTCTTTTTGTG
AGTTTTTTAACGTTTTGTGGCGCTGATCAGACACTTATTGAGAAGGAATTAACCGGAAGA
ACTGTTTATATCTCCAAAATTAAGCTAAATTGGAACGATGCCTTCGATTACTGCATCCGC
AATGGCCTCACCTTTGCTAAGATTAAATCAGCTGAAGAAAACACCGAACTGAGTGAGAA
ACTCAAGACAGTCATTCGTACGGAGGAGTTTCAAGTTTGGATTGGAGGCATTGAACATC
ATCAAGACAGTTCCTTCCGCTGGGTAAGCGACTCCCAACCAATAACCAACAAATTGGGC
TACAAATACACAAACTGGAATACCGGAGAGCCCACAAATTACCAAAACAACGAATATT
GCTTGGAAATATTATTCCGGAAGGAAGATGGAAAATGGAATGATTTTCCCTGCAGTGCA
AGACATCATTTTGTTTGTGAAAAAAGAACAAAATAAAATGAAGAAAATGTGATTTTCCT
TTGGTTGAAGAATAAAATTCTGTTGAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL17 (SEQ ID NO: 20)

ATTTAGTTTGTGTTTAACAAAACAAGAATGCAGAACTTCCTTTTAGTTTCCTTGGCTTTAG
CTGCCTTAATGCTATGTGCCGAAGCAAAGCCGTACGATTTTCCGCTTTATCAGGACTTAA
TTCAGGGCGTTATTCAGCGCGAAAGTCAAGCTGAGAGGGAGAAGAGAAGCCCCAATGA
GGACTATGAGAAGCAATTTGGGGATATTGTTGATCAAATTAAGGAAATTAGTTTCAATG
TCATGAAAATGCCCCATTTTGGAAGCTCTGATGATAATCGTGATGATGGCGAGTACGTTG
ATCATCATTATGGTGACGAAGATGATCGTGATTATGATCATTACTAAATACTACTTGCTC
CTGCTGAATGACTTGAAGGAATCATTTTTTTGCAAAAATATCCATCAAATTATTGAATTA
ATAAAGTTGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJM06 (SEQ ID NO: 22)

GTTTAAGGAATTTCTTTCATCTCAGTCTTCGATTTTCTTTAAACAAATAATGAAGTTTTAT
ATTTTTGGAGTTTTCCTGGTGAGCTTTCTTGCATTATGCAATGCTGAGGATTATGATAAA
GTAAAACTTACTGGAAGAACTGTTTACATCTCCAGATCAAAGGCTCCGTGGTTCACAGCT
TTAGACAATTGTAATCGTTTACGCTTCACCTTCGCCATGATCAAGTCTCAGAAGGAGAAT
GAAGAGCTAACAAATGCGCTTTTAAGTGTAATTAAATCTGACGAAGAAAATGTTTGGAT
TGGAGGTCTTAGGCACGATCTGGATGACTACTTCCGTTGGATTAGTTTTGGAACTGCATT
GTCAAAGACTTCGTACACCAATTGGGCCCCAAAGGAACCC
ACAGGAAGGCCCCATAGAACTCAAAATGATGAATTCTGCATGCAAATGTCTTTCAAAGA
TGGTGGCAAATGGAGTGATAACACCTGTTGGCGTAAACGTTTGTACGTTTGTGAAAAGC
GTGATTAAATAAAGGAACACTGCCAATGAATATTGGGCAATTTGAGAGAAATTAAATTA
AAAAAAAAAAAAAAAAAAA

LJM17 (SEQ ID NO: 24)

AGTCAGTGTTAATGAAGAAATTGCAATTATGAGGTTCTTCTTTGTTTTCCTTGCCATCGTC
CTTTTTCAAGGGATCCACGGAGCTTATGTGGAAATAGGATATTCTCTGAGAAATATTACA

TTCGATGGATTGGATACAGATGACTACAATCCAAAGTTCAACATTCCAACGGGTTTGGC
AGTTGATCCCGAAGGATATAGGCTCTTCATAGCCATCCCAAGGAGAAAGCCAAAGGTTC
CCTACACTGTGGCTGAACTGAATATGGTCATGAATCCCGGATTTCCCGTCGAGAGAGCTC
CGAGCTTTGAGAAATTCAAAAAATTCAATGGCGAGGGCAAAAAGGATCTTGTTAATGTG
TATCAGCCAGTCATTGATGATTGTCGTCGTCTTTGGGTGCTTGACATTGGGAAGGTGGAA
TACACCGGTGGTGATGCTGATCAATATCCCAAAGGAAAGCCTACCCTAATTGCCTACGA
CCTCAAGAAGGATCATACTCCGGAAATTCATCGATTTGAAATTCCAGACGATCTCTATAG
CTCACAAGTTGAATTTGGTGGATTTGCCGTTGATGTTGTTAACACGAAAGGAGACTGTAC
GGAGTCATTTGTCTACCTGACCAATTTCAAGGATAACTCTCTAATTGTCTACGATGAGAC
ACAAAAGAAAGCTTGGAAATTCACAGATAAAACATTTGAAGCTGATAAGGAATCCACGT
TCTCCTACTCGGGAGAGGAACAAATGAAGTACAAAGTCGGTCTTTTTGGGATAGCTCTG
GGTGATAGGGATGAAATGGGGCATCGTCCTGCCTGCTACATCGCTGGGAGTAGCACCAA
AGTCTACAGTGTTAACACTAAAGAACTCAAAACAGAGAATGGTCAGTTAAATCCTCAGC
TTCACGGTGATCGTGGAAAGTACACAGATGCAATTGCCCTAGCCTACGATCCTGAGCAT
AAAGTCCTCTACTTTGCTGAATCCGACAGCAGGCAGGTGTCCTGTTGGAATGTAAATATG
GAGCTAAAACCAGACAATACGGATGTGATCTTCTCTAGTGCCCGTTTTACTTTTGGAACG
GATATTTTGGTTGATAGCAAGGGAATGCTGTGGATAATGGCTAATGGACATCCACCAGT
AGAGGATCAAGAGAAGATTTGGAAGATGAGATTCGTAAACCGGAAGATCCGTATTATG
AAAGTGGATACGGAACGTGTTTTCAAATATTCACGCTGCAATCCAAATTATAAGCCCCC
AAAGGAAATTGAAGTTTGAGACACAGGAAAAAGCTCAATTTTCAACAAGAATTTGATCT
TAATCTGAATACCCTAAAGTCTGTCAAAGAATTTCATATTATTTGAAAACCAATAAATTG
ATTAATTTTCCGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL04 (SEQ ID NO: 26)

ACTAAAGCGTCTCACCGAAATCAGGGAAAATGATTAAGGAAGTTTTCTCTCTGGCTCTA
CTTGTGGCCTTGGCACAGTGTGCTAATGAAATCCCTATTAATCGTCAGGGGAAAGATTAT
CCAGTTCCGATCATTGATCCAAATAAATCATCTTCGGATGATTATTTCGATGATCGCTTC
TACCCTGATATTGATGATGAGGGCATAGCTGAGGCTCCTAAGGATAATAGGGGAAAATC
CCGTGGTGGTGGTGCGGCTGGCGCAAGAGAAGGTAGGTTAGGTACGAATGGGGCTAAA
CCGGGTCAGGGTGGAACTAGACCAGGACAGGGTGGAACTAGGCCAGGACAGGGTGGAA
CTAGGCCAGGTCAGGGTGGAACTAGGCCAGGTCAGGGTGGAACTAGACCTGGGCAAGG
TAGAACTAAGCCTGCTCAGGGAACTACTAGGCCAGCTCAGGGAACTAGAAATCCAGGAT
CGGTTGGTACGAAAGAAGCCCAGGATGCGTCAAAACAAGGTCAAGGTAAAAGAAGGCC
AGGGCAAGTTGGTGGTAAAAGACCAGGACAAGCAAATGCTCCTAATGCAGGCACTAGA
AAGCAACAGAAAGGCAGTAGAGGCGTTGGAAGGCCTGATCTATCGCGCTACAAAGATG
CCCCTGCTAAATTCGTTTTCAAATCTCCCGATTTCAGTGGAGAAGGCAAAACTCCAACTG
TAAATTACTTTAGAACGAAGAAGAAGGAGCACATTGTGACCCGTGGTAGTCCTAATGAT
GAATTTGTTCTGGAGATTCTCGATGGGGATCCAACTGGGCTTGGACTAAAGAGTGAAAC
CATAGGCAAAGATACGCGTTTAGTGCTGGAGAATCCTAATGGAAATTCCATCGTGGCTC

GTGTTAAGATCTACAAGAACGGTTATTCAGGATGAAGAAGAAATCCTTTGATTTCCCCCC
CCCCCTCTTCCTTTAAAATTCAACATAATAAAAAAAAAAAAAAAAAA

LJM114 (SEQ ID NO: 28)

GTCTTTTCCTGAGTGTTTCATTAACAAAATGAATTCAGTAAACACTTTAATTTTAACTCTT
CTATTTGCAATTTTTTTATTAGTGAAAAGGTCTCAGGCTTTTCTTCCATCTGACCCAAGTA
TCTGTGTTAAAAATTTAGTATTGGATACAGGAAGGACTTGTGAGGAAAGTGAATATTTTC
CGGATATCAAGAACGTTAAAAATGGAAAAAGAGTTTACATTGTCTGCACTGATTCAGAT
GCAGTTGATTATAAATTTTATATTTGTTTCGATATGAATCGTCTTTCTGGACCACCGTATC
CTGAGGAAGAAATCCTTCGTGAATCAACGGTAACTTATGCCCAAATTTATGAGCTGATG
ACTACGGAAACCACTGAAACCAAAAAGCCAAAAAAGAAACCAAAGAATTCAAAAACGG
ACCCAGACCCTCCAGCAATTCGTCCAGGATTTTCATTTAGAAATTCAATTTCTGTTTAATT
TTACAATTTATTTTGAAAGAAAAATGATATTTCGAAATATTCTATACAAAAAAACAACA
GTTATAAAACGAAAATTCAATCATTTCAATGAGAAAACTTAGTCTTGAGTAAGGTTTATT
CACCACCCGACGCCACGCTATGGTGAATAATTTTCTTTATTCACCACATCAAAATGACGG
CTTATAAACTTCAACAAATAGTTTGGAAAATACATTTCTAACTAATGCAATGTTTACTTA
AAATCACTTTACAAATTCACGCATTTGAGATGCAACAAATATATACAATTCAACGATAT
AAACTTTCCACAAGGAAAACTTTCAACCAAAAAAAAAAAAAAAAAAAA

LJM111 (SEQ ID NO: 30)

ATCATTCAAAAGGCAGCAGCACAATGAAGTTATTTTTCTTTCTTTACACTTTTGGTCTAGT
CCAAACGATTTTTGGAGTAGAAATTAAACAAGGATTTAAATGGAATAAAATCCTTTATG
AGGGCGATACATCAGAAAACTTCAATCCAGATAACAACATCCTTACGGCTTTTGCGTAC
GATCCTGAGAGTCAGAAACTCTTCCTAACTGTCCCGAGGAAATATCCCGAAACTATGTA
CACTTTGGCAGAAGTTGATACTGAGAAAAATTCTTTTGAATCGGGAGATACTTCCCCGCT
CCTTGGAAAATTCAGTGGTCATGAAACTGGGAAAGAACTTACATCAGTTTATCAGCCAG
TTATCGATGAATGTCATCGTCTTTGGGTTGTTGATGTTGGATCAGTAGAACGTAACTCAG
ACGGCACAGAAGGTCAGCCAGAACATAATCCTACCCTTGTGGCGTACGATCTCAAAGAA
GCCAACTATCCTGAAGTTATTCGTTACACGTTTCCCGATAATTCCATTGAGAAGCCCACA
TTTCTGGGTGGATTTGCCGTTGATGTTGTAAAGCCGGATGAATGCAGTGAAACTTTTGTC
TACATCACAAACTTCCTCACCAACGCCCTCATAGTATACGATCATAAGAATAAGGACTC
CTGGACGGTACAAGATTCAACTTTTGGACCAGATAAAAAGTCAAAGTTTGACCACGATG
GACAACAGTATGAATACGAAGCAGGAATCTTCGGGATTACCCTTGGAGAGAGAGATAA
CGAAGGAAATCGTCAAGCGTACTATTTAGTAGCAAGTAGTACCAAACTTCACAGCATCA
ACACCAAAGAACTGAAGCAAAAAGGAAGCAAAGTTAATGCAAATTATTTGGGAGATCG
TGGTGAATCCACCGATGCCATAGGCTTAGTTTACGATCCAAAAACCAAAACTATCTTCTT
CGTTGAGTCAAATAGCAAAAGAGTATCATGCTGGAATACCCAGGAAACACTAAACAAG
GATAAAATTGATGTAATCTATCACAATGCAGACTTTTCCTTTGGAACAGATATATCGATT
GATAGTCAGGATAATTTGTGGTTCCTAGCAAATGGACTTCCACCTCTGGAAAATTCTGAT

AAATTTGTCTTTACAAAGCCACGTTATCAAATATTCAAAGTCAACATTCAAGAAGCAATT
GCTGGAACTAAATGTGAAAAGAATCTTTAACAAATGAAACTTTGTAGAAAAATACATAA
TATCTGAATAAAAAGTCATAAATGTACCATAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAAAAAA

LJM78 (SEQ ID NO: 32)
CTTTAAAGCAAAAATTTTGTGGGAAAGGAAGTTACCCGGAGATGACGTTTCTAATTATA
CTTGGTGCATTTCTCCTTGTTCAAATTATTACAGCTTCAGCTTTAGGATTGCCTGAACAGT
TTAAAGGTTTAGAGGATTTACCTAAAAAACCTTTGGCAGAGACTTATTATCACGAAGGA
TTGAATGATGGAAAAACGGATGAAATGGTGGATATTTTTAAAAGTCTTAGCGATGAATT
TAAATTCAGTGATGAAAATTTAGATGTTGGTGAGGAGAAAAATTACAAGAAACGTGATA
TAACCCAAAATTCAGTGGCAAGGAACTTCCTATCAAACGTAAAGGGAATTCCTTCAATG
CCATCACTCCCTTCAATGCCTTCAATGCCATCAATTCCTTCACTTTGGTCAAGTCAGACA
CAGGCGGCACCAAATACCGCACTTGCCCTTCCTGAATCTGATTATTCCCTTCTAGATATG
CCGAATATTGTGAAAAATTTCCTAAAGGAAACAAGAGACCTCTATAACGATGTTGGAGC
TTTTCTTAAGGCAATTACAGAAGCTTTAACAAATAGATCTTCATCATCTCAACTTCTTTCC
TCCCCAATGGTGAGCACGAATAAAACCAAAGAATTTATTCGGAATGAAATACAAAAAGT
CCGAAAAGTGAGAAATTTCGTCCAGGAAACTCTTCAGAAAATCCGAGACATTTCTGCTG
CTATTGCCAAAAAGGTAAAATCATCAGAATGTCTGTCCAATCTTACGGACATCAAAGGA
CTTGTATCAGACGGAATTAATTGTTTAAAGGAAAAATTCAATGATGGAAAACGAATTAT
CCTGCAATTGTACAATAATTTACTAAAAGGACTCAAAATTCCAAATGACCTAATGGTTG
AATTGAAGAAATGTGATACAAATCAAAACAATACTTTGGGAAGAATAATCTGTTATTTT
TTGACACCATTGCAACTGGAAAAAGAACAAATTCTTCTACCTGTAGAATTTATAAAGCG
CATTCTTGAATTAACCCACTACTTTTCCACAATGAAAGAAGATCTTATCAACTGTGGCAT
CACAACGATTGCATCCATTACGTAAAAAATGGAAAAATGTGCCGGTGAAATGCTTGAAA
TCACCAAAGAAATTTCATCGCAAATAACAGTTCCAGAATAACCAAATTTTAATGATTACT
TCTCAAGGAAAATACTACCAAAAGGCATTAATTAAAACGATGTTTTTTATAAACAATGT
AAGAAAAAAAAAAAAAAAAAAAAAAAAA

LJS238 (SEQ ID NO: 34)
AGTTAATCTTCTGTCAAGCTACAAAAATGCTTAAAATCGTTTTATTTCTATCAGTTTTGGC
TGTATTAGTGATTTGTGTAGCAGCAATGCCAGGATCCAATGTTCCTTGGCACATTTCACG
AGAAGAGCTTGAGAAGCTTCGTGAAGCTCGAAAGAATCACAAGGCACTCGAGAAGGCA
ATTGATGAATTAATTGACAAATATCTCTGATTTTGAAGAGCAAGGAAGAGGAAATAAAC
GGCCGAGGAAGGATTTTCTTTAGAGATTCTTCTTTTTATTACTTCAAACCTAACTTCAAA
ATCAGTCTGATATTTTTTTAATTTGAAAAAAATATTGAAAATTTTAACTATTTGTGAAATT
TAAATAAATAAAGAATGTCAGAAGCAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJS169 (SEQ ID NO: 36)

AATTTTCACCATGAAGTTTTCTTGCCCAGTTTTCGTTGCAATTTTCCTTTTGTGCGGATTTT
ATCGTGTTGAGGGGTCATCACAATGTGAAGAAGATTTAAAAGAAGAAGCTGAAGCTTTC
TTTAAGGATTGCAATGAAGCAAAAGCCAATCCTGGTGAATACGAGAATCTCACCAAAGA
AGAAATGTTTGAAGAATTGAAAGAATATGGAGTTGCTGACACAGACATGGAGACAGTTT
ACAAACTTGTGGAAGAATGTTGGAATGAATTAACAACAACGGATTGTAAGAGATTTCTC
GAAGAGGCTGAATGCTTCAAGAAGAAGAATATTTGTAAATATTTCCCAGATGAAGTGAA
ATTGAAGAAGAAATAAATTTTTAGCTTGAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAA

LJL11 (SEQ ID NO: 38)

AGTTGCAAGAATTTCTTCATTGCGTTAAGATGTTGTTTTTCCTTAACTTTTTTGTGCTGGT
GTTCAGCATAGAACTGGCGTTGTTAACAGCATCAGCAGCAGCAGAAGACGGCAGCTATG
AGATCATAATTCTTCACACCAATGATATGCACGCGCGTTTTGATCAAACCAATGCTGGAA
GCAACAAATGCCAAGAAAAAGACAAGATTGCTTCCAAATGCTACGGAGGATTTGCAAG
AGTTTCAACAATGGTGAAAAAATTCCGAGAAGAAAATGGCAGCAGTGTCTTGTTCTTGA
ATGCTGGTGACACGTATACAGGTACCCCATGGTTTACCCTCTACAAGGAGACCATTGCA
ACGGAGATGATGAACATCCTTCGTCCAGATGCAGCCTCACTGGGAAATCATGAATTCGA
CAAAGGAGTAGAAGGACTCGTGCCATTCCTCAATGGTGTCACCTTCCCTATTTTAACAGC
GAATTTGGACACTTCTCAAGAGCCAACAATGACCAATGCTAAAAATCTCAAACGCTCAA
TGATTTTTACGGTTTCCGGGCACAGAGTTGGTGTAATTGGCTACCTAACGCCTGATACAA
AATTCCTCTCGGACGTTGGTAAAGTTAATTTTATTCCGGAAGTTGAAGCCATCAATACGG
AAGCACAGCGTCTGAAGAAAGAGGAAAATGCCGAAATAATCATCGTTGTTGGACATTCA
GGGTTGATAAAAGATCGAGAAATTGCAGAGAAATGCCCACTGGTTGACATAATTGTTGG
AGGACATTCACACACATTCCTCTACACAGGAAGTCAGCCTGATCGTGAGGTTCCTGTAG
ACGTTTATCCTGTTGTTGTGACCCAATCCAGTGGGAAGAAAGTTCCAATTGTTCAAGCCT
ATTGCTTTACAAAGTATTTGGGGTACTTTAAAGTGACGATCAACGGAAAAGGAAATGTT
GTGGGATGGACTGGGCAGCCAATTCTCCTTAATAACAACATTCCCCAAGATCAGGAAGT
TCTCACTGCTCTTGAAAAGTACAGAGAACGCGTGGAAAACTATGGAAATCGCGTAATTG
GAGTTTCCCGTGTAATTCTCAATGGGGGGCATACTGAATGTCGTTTCCATGAATGCAATA
TGGGTAATCTCATCACGGACGCTTTTGTGTATGCCAATGTAATCAGTACACCAATGAGTA
CGAATGCCTGGACAGATGCAAGTGTTGTTCTGTATCAAAGTGGTGGCATTCGTGCCCCA
ATTGATCCTCGTACCGCGGCAGGGAGCATCACACGCCTCGAGTTGGACAATGTTCTACC
ATTTGGGAATGCACTGTACGTCGTAAAAGTTCCTGGGAATGTCTTACGCAAAGCTTTGGA
ACATTCAGTTCATCGATACTCCAACACTTCGGGATGGGGAGAATTTCCACAAGTTTCGGG
GCTAAAGATTCGTTTTAACGTCAATGAAGAAATTGGAAAACGCGTAAAGTCCGTTAAAG
TTCTCTGTAGCAATTGCTCTCAACCTGAATACCAACCACTGAGAAATAAAAAAACTTAC
AACGTTATCATGGACAGTTTTATGAAGGATGGAGGTGATGGGTATAGCATGTTCAAGCC

CTTGAAGATCATCAAGACCCTCCCACTGGGAGATATTGAAACAGTAGAAGCTTATATTG
AGAAAATGGGCCCCATTT
TCCCAGCAGTCGAGGGAAGGATCACTGTTCTTGGGGGACTTCAAAAATCAGATGAGGAT
TGGCATTAGAAACATCCTGGACGTTATGGAAAGAATAAAAGAAGGATCATAGAAAAAA
AAAAAAAAAAAATAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL08 (SEQ ID NO: 40)
GTCAGTGATCTGATAAGTTATTAAAATGAAGCAAATCCTTCTAATCTCTTTGGTGGTGAT
TCTTGCCGTGCTTGCCTTCAATGTTGCTGAGGGCTGTGATGCAACATGCCAATTTCGCAA
AGCCATAGAAGACTGCAAGAAGAAGGCGGATAATAGCGATGTTTTGCAGACTTCTGTAC
AAACAACTGCAACATTCACATCAATGGATACATCCCAACTACCTGGAAATAATGTCTTC
AAAGCATGCATGAAGGAGAAGGCTAAGGAATTTAGGGCAGGAAAGTAAGAGATTGAGG
AAAATTGTAGCCGAAGAGAGAAGGAAGGAAAGTCCCATATTTTGTTTGTTAATTGTAAC
GAATTTTGCGAAAAAAATAAAATATTATGCACTCCAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAA

LJS105 (SEQ ID NO: 42)
TATTTTTAATAATTCTGTGTAAAATGAACGTTCTTTTCGTGTCTTTCACGCTCACAATTCT
TCTTCTCTGTGTTAAGGCACGGCCAGAAGATTTCGTAGCTCTTCAGGATCAAGCTAATTT
CCAGAAATGCCTCGAACAATATCCAGAACCAAATCAATCTGGAGAAGTTCTTGCGTGCC
TCAAGAAGCGCGAAGGTGCCAAAGATTTCCGGGAAAAGAGGAGCCTGGATGACATAGA
AGGGACTTTCCAAGAGTCTGGAAATCTCTGGGGTGCATAGGAAGCTCAGAGGACTTCTA
ATCAATCTGTGAGAAGAGAACCCAACGGCTAGAGAAAATTTAAGGAAAATAAAGAAAT
TAATGAAGCATTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
AAAAAAAAAAAAAAAAA

LJL09 (SEQ ID NO: 44)
GTATATCAAGTATCATTCAAGTGAATCATTGGCTCCGTAATTTGTACAAAAGAAAAAAA
AAGTTGATAAAATCATGAAAATCACTGTGATTTTATTCACGGGATTTACAATTGCCCTCG
TGAGTAGTGCTGTGCTTAAGAAAAACGGTGAAACTATTGAAGAAGAAGAAGTAAGAGC
TGAGCAACGACTTAGAGAGATCAATGAGGAACTTGATCGTAGGAAGAATATCAATACTG
TAGCCGCTTGGGCTTATGCATCCAATATTACTGAGGTCAATCTCAAGAACATGAATGATG
TGTCGGTTGAAACCGCGAAATACTACAAGGAACTTGCATCTGAATTGAAGGGATTCAAT
GCCAAGGAATACAAGAGTGAGGATCTGAAGAGACAAATTAAGAAGCTAAGCAAGTTGG
GATATAGTGCTTTACCATCTGAGAAGTATAAGGAGCTTTTGGAAGCTATCACATGGATG
GAATCGAATTATGCAAAAGTGAAAGTTTGCTCATACAAGGATCCAAAGAAATGTGATTT
AGCACTTGAACCTGAAATTACGGAAATCCTTATTAAAAGTCGAGATCCTGAGGAACTTA
AATATTATTGGAAACAATGGTACGACAAAGCTGGCACACCAACTCGAGAGAGTTTTAAT
AAGTATGTACAACTAAATCGTGAAGCAGCGAAATTGGATGGATTTTATTCGGGTGCAGA

ATCTTGGCTTGATGAATATGAAGATGAGACATTTGAGAAACAACTTGAGGATATCTTCG
CCCAAATTCGCCCACTGTACGAGCAACTCCATGCTTATGTTAGATTCAAGCTGAGGGAA
AAGTATGGAAATGACGTTGTTTCGGAGAAAGGTCCCATTCCAATGCATCTCTTGGGGAA
CATGTGGGGTCAAACGTGGAGTGAAGTTGCCCCAATTTTAGTCCCATACCCCGAAAAGA
AGCTCCTCGATGTTACCGATGAGATGGTTAAGCAGGGATACACACCAATTTCTATGTTTG
AAAAAGGAGACGAATTTTTCCAAAGCTTGAATATGACGAAACTTCCAAAAACCTTCTGG
GAGTACAGTATTTTGGAAAAACCCCAAGATGGTAGGGAATTGATCTGCCATGCAAGTGC
ATGGGACTTCTATACAAAGGATGATGTAAGGATTAAACAGTGTACCAGAGTTACAATGG
ATCAATTCTTCACGGCTCATCATGAGCTTGGTCACATTCAATATTATTTGCAATATCAAC
ATTTGCCGAGTGTTTACAGAGAAGGTGCCAATCCAGGCTTTCACGAGGCTGTTGGGGAT
GTTCTCTCTCTTTCGGTATCAAGTCCTAAACATTTGGAAAAAGTTGGTTTGCTTAAAGAC
TTCAAATTTGATGAAGAATCCCAGATAAATCAACTTCTAAATTTAGCTCTGGATAAAATG
GCATTCCTCCCATTTGCCTATACCATTGATAAATATCGCTGGGGTGTGTTTCGGGGTGAA
ATTTCGCCGTCTGAGTACAATTGCAAATTTTGGGAAATGCGTTCCTACTATGGTGGTATA
GAACCACCAATTGCACGTTCTGAGAGTGATTTTGATCCACCAGCAAAATATCATATTTCA
TCGGATGTTGAGTACCTCAGGTATTTGGTTTCCTTCATTATTCAGTTCCAATTCCATCAAG
CTGTGTGCCAAAAGACTGGTCAGTTCGTACCGAATGATCCGGAGAAGACTCTTCTAAAT
TGTGACATCTACCAGAGTGCTGAGGCTGGTAATGCCTTCAAAGAAATGCTCAAATTGGG
ATCCTCAAAACCATGGCCAGATGCAATGGAAATTCTTACGGGGCAAAGGAAAATGGATG
CTTCTGCATTAATTGAGTACTTCCGTCCACTCAGTGAGTGGTTGCAGAAGAAGAATAAG
GAACTAGGAGCTTATGTTGGCTGGGACAAATCTACTAAGTGTGTCAAAAACGTCAGTTA
ATTTTTTGTGAGCCCTAAAAAATATTCATAACATTTCAATATGACAAAATATATGATTTT
CGTGAAAACTAAGCATGAGTAAGTTTTTTTTGTGAATTTTTAGCAGTTTCATTTCAGAAT
AAACGTCAAATTTTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL38 (SEQ ID NO: 46)

TCAGTTAGTTGACTAACAAACCACAATAGAGACACTAAAATGAAGACATTCGCCTTAAT
CTTCTTGGCTCTTGCTGTTTTTGTGCTCTGCATTGACGGAGCTCCAACTTTTGTGAATTTA
CTGGACGACGTACAGGAAGAGGTAGAAGTTAATACGTATGAGCCTTAGGAAGAAAATG
TTTGAGGAGTTTCAGGCAGAGGCAGAGCTTTCCCAGAGAGGGAGCTTTTGCCTTGCTGT
AGATTTTTAAAAATGAATCAATTTGATTGGAGCAATTACGCTATATTTGTGGGAATATTT
TTGAATTAAAAACTAATTATGGAAATTAATATATAATTTTCAGAATTTCAATAAATTCAT
CAAAATTGTATTAATTAAAAAATATTGTATGAAATTCCCAATAAAAGCTTTCAAATTAAA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJM04 (SEQ ID NO: 48)

GGCCATTATGGCCGGGGATAGAACTTAATTGTTGTTAAAATGAATCACTTGTGCTTTATT
ATTATTGCTCTATTCTTTTTGGTTCAACAATCTTTGGCTGAACATCCAGAAGAAAAATGT
ATTAGAGAATTGGCGAGAACTGATGAAAACTGCATTCTTCATTGTACGTATTCGTACTAC

GGATTCGTTGATAAAAATTTCAGGATCGCTAAAAAACATGTTCAAAAATTCAAAAAAAT
CCTAGTTACATTCGGCGCTGTTCCTAAGAAAGAAAAAAAGAAACTTTTAGAGCACATTG
AGGCTTGTGCGGATTCTGCGAATGCTGATCAACCTCAAACTAAAGATGAAAAATGTACA
AAAATAAATAAGTACTATCGTTGTGTTGTGGATGGAAAAATATTACCCTGGAATAGTTA
TGCTGATGCAATCATTAAGTTTGATAAAACCCTTAACGTATGAAGCAAAGATATTCGAA
AAAAAAACATCAAGATTATGCTGGAAAGAAAAAAATAAAAAAAAAATTGTGCTAATCAA
ATTGAATTAACGCTTAATGCTATATTAAAAAAAAAAAAAAAAAAAA

LJM26 (SEQ ID NO: 50)

GTCGGAGATCGTCTGCCTTGATGATCACATCGTGATTGTGAGTTACAAGAGTGAAACTTT
TTAAGTGTGTGTGTCTTAGCAAAGTGATTTCCACAATGAAGATTATTTTTTTAGCCGCTTT
TCTACTAGCGGATGGTATTTGGGCTGCTGAAGAACCTTCAGTGGAAATTGTAACACCAC
AATCAGTGCGGAGACACGCTACGCCAAAAGCCCAGGACGCGAGGGTAGGAAGTGAATC
CGCAACAACAGCACCAAGACCAAGTGAATCAATGGATTACTGGGAGAATGATGATTTCG
TCCCATTTGAGGGTCCATTCAAGGATATTGGAGAATTCGACTGGAACCTTTCGAAGATCG
TTTTTGAGGAAAACAAAGGTAATGCCATCTTGTCGCCACTCTCTGTGAAGCTACTAATGA
GTTTGCTCTTCGAGGCCAGTGCGTCAGGTACCTTGACCCAGCACCAACTCAGACAAGCC
ACTCCCACCATCGTCACCCACTATCAGTCTCGAGAATTTTACAAGAATATCTTTGACGGT
CTCAAGAAAAAGAGTAACGACTACACGGTTCACTTTGGTACGAGAATCTACGTGGATCA
GTTTGTGACGCCTCGCCAGAGATATGCTGCCATTTTGGAGAAGCATTATCTGACTGATCT
CAAAGTTGAGGACTTCTCGAAGGCAAAAGAAACAACTCAGGCAATCAATAGTTGGGTGT
CAAACATCACAAATGAGCACATAAAGGATCTCGTGAAGGAGGAAGATGTTCAGAATTC
AGTTATGCTCATGCTTAATGCAGTCTACTTCCGCGGACTCTGGCGCAAGCCTTTCAATCG
TACACTCCCACTGCCCTTCCACGTGAGCGCTGATGAGTCCAAGACGACTGATTTTATGCT
AACCGATGGGCTCTACTACTTCTACGAGGCAAAGGAATTGGATGCTAAGATCCTCAGAA
TTCCTTACAAAGGTAAACAATACGCAATGACTGTGATCTTACCAAATTCCAAGAGTGGC
ATTGATAGCTTTGTGCGTCAGATTAACACGGTCCTCCTGCACAGGATTAAGTGGTTGATG
GATGAAGTGGAGTGCAGGGTTATTCTACCCAAGTTCCACTTTGACATGACGAATGAGCT
GAAGGAATCGCTCGTAAAGTTGGGCATCAGTCAGATTTTCACATCAGAGGCATCTTTGC
CATCATTAGCACGAGGACAGGGCGTACAGAATCGTCTGCAGGTGTCTAATGTGATTCAG
AAGGCGGGAATAATTGTGGATGAGAAGGGCAGCACAGCCTATGCTGCGTCAGAAGTGA
GCCTAGTCAACAAGTTTGGAGATGATGAGTTCGTCATGTTCAACGCTAATCATCCATTCC
TCTTTACAATTGAGGACGAAACCACCGGCGCAATCCTATTTACGGGAAAAGTCGTCGAT
CCCACGCAATAGGGAATGAAAAGCATTTCATCGTATACAACTTTTTTTTTAATTAATTAT
TCCTCATTGAAGGACATTAATAGAGCATCTTCTCAGGAAGGCACTCCTGACTTATTTTTA
CTAAATGTGATCCTTGGACACATAAAAAAAACAGCTGTACTTTCTACTTTTTATAATATA
CGACCATATTTGTGAGGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJS03 (SEQ ID NO: 52)

TCAGTTAAGCAGATTTTCAAGCTAAAGAAACTTAACTAAGATGCGATTCCTTCTTTTGGC
CTTCTCCGTTGCTTTGGTGCTTTCACCAACATTCGCCAAACCAGGTCTTTGGGACATTGTA
ACTGGTATTAATGATATGGTAAAAAATACTGCGAATGCACTCAAAAATCGTCTAACAAC
TTCTGTGACATTATTCACAAATACCATCACCGAAGCTATAAAAAATGCAAATTCTTCTGT
TTCGGAACTCCTTCAGCAAGTCAATGAAACCCTTACGGATATTATTAATGGTGTAGGACA
AGTGCAGAGTGCCTTTGTGAATTCAGCTGGAAATGTTGTTGTGCAAATTGTTGATGCCGC
TGGAAATGTTTTGGAAGTTGTTGTTGATGAGGCTGGAAATATCGTGGAGGTAGCTGGAA
CAGCATTGGAAACTATCATTCCACTGCCCGGTGTAGTGATTCAGAAGATAATTGATGCTC
TCCAAGGAAATGCAGGGACTACATCGGATTCAGCTTCATCAACTGTGCCCCAACAATCT
TAACTACAACCGCAATGATGTTGTCTTTAACGGAGAATTTTTAAATTTGAATATCAAAAT
CCAAGATGAAATATTCAGATTTTTCAATCAATATGATACGAAATTTTGAAATTATTTTTC
CGACTAAAGCAATTTGTAAAAGGAAAACCAAATAAATATTTGAAATTGTAAAGAAAAA
AAAAAAAAAAAAAAAAAAAAAAA

LJS192 (SEQ ID NO: 54)

ATATCAATTTTATCATCATGGTGAAGTACTCGTGTCTTGTTCTTGTTGCAATTTTTCTTCT
GGCCGGACCCTACGGCGTTGTAGGTTCTTGTGAGAATGACCTGACAGAGGCCGCCAAGT
ATCTTCAAGATGAATGCAATGCAGGTGAAATTGCAGATGAATTTCTACCCTTCTCTGAAG
AAGAAGTGGGTGAAGCATTGAGCGACAAACCAGAAAACGTGCAGGAAGTCACCAACAT
CGTGAGAGGATGCTTTGAAGCTGAACAAGCCAAAGAGCATGGAAAATGTGAAAGATTTT
CCGCTTTGAGTCAATGCTACATTGAAAAGAATTTATGTCAATTCTTCTAAAATATTTTGA
AGAAAAGTTATGAATGAAAATTTTCTGAAATTTTGTTGCAAAAATATATAAATTGCCCA
ATTAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJM19 (SEQ ID NO: 56)

AGTTTAATTTTCATCATGAAGTTCTTCTACTTGATTTTCTCTGCAATTTTCTTTCTGGCTGA
TCCTGCTTTGGTCAAGTGTTCAGAGGATTGTGAGAATATTTTTCATGACAATGCGTACCT
CCTTAAATTGGATTGTGAAGCAGGAAGGGTTGATCCTGTTGAATACGACGATATTTCGG
ATGAAGAAATATATGAAATAACGGTCGATGTTGGAGTTTCATCTGAGGACCAGGAGAAA
GTTGCGAAAATAATAAGGGAGTGCATTGCACAAGTTTCAACGCAAGATTGCACGAAATT
TTCAGAAATTTATGATTGTTACATGAAGAAGAAAATCTGTAATTATTATCCTGAAAATAT
GTAAAAAAAAATTATTTATTTATATAAAAAAATATAAGGATTAAAATCTCTTATTGATTG
TAAAAATGGCCTAATATTGAAGCAAAAATTAAAGCATGAAACAAGACCAAAAAAAAAA
AAAAAAAAAAAAAAAAAAA

LJL138 (SEQ ID NO: 58)

TCAATCTAACAATGCACCTGCAATTGAATTTGTGCGCTATTCTCCTTTCGGTACTAAATG
GAATTCAGGGCGCTCCCAAAAGTATTAATTCAAAATCCTGCGCAATCTCCTTTCCGGAGA

ATGTAACGGCTAAGAAGGAGCCAGTGTACTTGAAACCATCAAATGATGGCTCATTGAGT
ACCCCCCTACAGCCAAGTGGGCCATTTGTAAGTCTCAAAATTGGAGAATCTCTTGCAATC
TTCTGTCCAGGTGATGGAAAGGACGTAGAGACAATTACGTGCAATACAAATTTCGATTT
AGCTTCATATTCGTGCAACAAGAGCACATCAACGGATACCATTGAAACGGAAGAAGTTT
GCGGAGGAAGTGGAAAAGTGTACAAAGTTGGTTTTCCGCTGCCCTCTGGGAATTTCCAT
TCAATCTACCAAACGTGTTTTGATAAGAAAAATCTCACACCTCTCTACTCAATTCACATT
CTCAATGGTCAAGCTGTTGGATATCACCTTAAGCACACAAGAGGAAGCTTTCGTACCAA
TGGTATCTACGGGAAAGTCAACATTGATAAACTCTACAAGACGCAAATTGAGAAATTCA
ACAAACTTTTCGGCCCTAAACAAACATTTTTCCGTAGACCCCTCAATTTTCTATCACGTG
GACACTTAAGCCCCGAAGTGGACTTTACATTCCGTAGGGAACAACATGCAACGGAAATG
TACATTAACACAGCACCACAGTACCAATCAATTAATCAAGGAAATTGGCTACGTGTTGA
AAATCACGTGAGGGATCTCGCAAAAGTTCTGCAGAAGGACATAACAGTCGTTACGGGAA
TTTTGGGGATACTTCGGTTGAAGAGTAAGAAAATAGAGAAAGAAATCTATTTAGGAGAT
GACGTAATTGCCGTACCAGCAATGTTCTGGAAGGCTGTTTTTGACCCTCAAAAACAAGA
AGCAATTGTCTTTGTTTCCTCAAATAATCCCCACGTGAAGACCTTTAATCCCAACTGCAA
GGATGTATGCGCTCAAGCTGGATTTGGGAATGATAATCTTGAATATTTCTCCAATTATTC
TATTGGTCTGACTATTTGTTGCAAACTTGAGGAATTTGTTAAAAGAAATAAAATAATTCT
ACCCAAAGAAGTAAATAACAAAAACTACACCAAAAAACTCCTTAAGTTTCCTAAAACAA
GAAACAAGGAGGGAGATAAGAAGGTGGTACGTAAGCGCGCCAAAGGAGCATAAATATT
AAACGAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL15 (SEQ ID NO: 60)

GTTCTACGATAAAATTTTCTTTTCAAACTTTTCTTTTAAAGAAAAATCTTCAAAAAGTTA
AAATGAATTTGCACCTTGCGATTATCCTCTTTGTGAGTTACTTCACACTGATCACTGCTAC
GGATCTAATTGAAAAGGAACTTTCTGATTGCAAAAAGATCTTCATCTCCAAGGCTGAGC
TAACTTGGTTCCAAGCTCTCGATTTCTGTACCGAACAAAACCTAACTTTGCTCTCAATTA
AATCCGCCCGGGAAAATGATGAGGTGACTAAAGCAGTTCGAGCTGAGGTTCATCTTCCA
GACACAAAGAAGTCTCACATTTGGCTCGGAGGTATTCGTTATGATCAAGACAAGGATTT
CCGTTGGATAAGCGATGGAACAACTGTTACGAAGACAGTCTACATCAATTGGTACCAAG
GAGAACCAAATGGTGGGAGGTACCAAAAGGAATTTTGTATGGAATTGTACTTTAAAACT
CCAGCTGGTCAATGGAATGATGATATTTGTACAGCAAAGCATCATTTTATATGTCAGGA
GAAAAAATAAATTGAATTGTTCATGTGTCTTTGGCGGTGCGAAGGTATAATTCAGGTTG
ACGACATAAATTGATTTTTCTTTCATTAAGAAAATAAAGGCTTGAATTTATAAAAAAAA
AAAAAAAAAAAAAAAAAA

LJL91 (SEQ ID NO: 62)

GTTCTACGATAAAATTTTCTTTTCAAACTTTTCTTTTAAAGAAAAATCTTCAAAAAGTTA
AAATGAATTTGCCCCTTGCGATTATCCTCTTTGTGAGTTACTTCACACTGATCACTGCTGC
GGATCTAACTGAAAAGGAACTTTCTGATGGCAAAAAGATCTTCATCTCCAAGGCTGAGC

TAAGTTGGTTCGATGCTCTCGATGCCTGTACCGAAAAAGACCTAACTTTGCTCACAATTA
AATCCGCCCGGGAAAATGAGGAAGTGACTAAAGCAGTTCGAGCTGAGGTTCATCTTCCA
GACACAAAGAAGTCTCACATTTGGCTCGGAGGTATTCGTTATGATCAAGACAAGGATTT
CCGTTGGATAAGCGATGGAACAACTGTTACGAAGACAGTCTACATCAATTGGTACCAAG
GAGAACCAAATGGTGGGAGGTACCAAAAGGAATTTTGTATGGAATTGTACTTTAAAACT
CCAGCTGGTCAATGGAATGATGATATTTGTACAGCAAAGCATCATTTTATATGTCAGGA
GAAAAAATAAATTGAATTGTTCATGTGTCTTTGGCGGTGCGAAGGTATAATTCAGGTTG
ACGACATAAATTGATTTTTCTTTCATTAAGAAAATAAAGGCTTGAATTTAGCAAAAAAA
AAAAAAAAAAAAAAAAAAA

LJM11 (SEQ ID NO: 64)

TTGAATTGAAGCAGCAGCAATGAAAGTGTTTTTCTCAATTTTTACGCTCGTCCTCTTCCA
AGGGACCCTTGGAGCGGATACTCAAGGATATAAATGGAAGCAATTGCTCTACAATAATG
TTACACCAGGATCCTACAATCCGGATAATATGATCAGTACGGCTTTTGCCTACGATGCTG
AGGGTGAAAAACTCTTCCTAGCTGTCCCAAGGAAGTTACCCAGAGTTCCGTATACATTG
GCGGAAGTGGATACAAAGAATAGTCTTGGTGTTAAGGGAAAACATTCACCGTTACTTAA
CAAATTCAGTGGGCACAAAACTGGGAAGGAACTAACATCAATCTATCAGCCAGTTATTG
ATGATTGTCGTCGCCTTTGGGTGGTTGATATTGGTTCCGTGGAATATCGCTCAAGAGGTG
CCAAAGACTACCCGAGTCATCGTCCTGCAATTGTTGCGTACGACCTAAAGCAACCAAAC
TACCCCGAAGTTGTTCGATACTATTTCCCCACAAGATTAGTGGAGAAGCCAACATATTTC
GGTGGATTTGCCGTTGATGTTGCAAACCCAAAGGGGGATTGTAGTGAAACTTTTGTCTAC
ATTACAAACTTCCTCAGGGGAGCTCTCTTTATATACGATCATAAGAAGCAGGATTCGTGG
AATGTAACTCATCCCACCTTCAAAGCAGAACGACCCACTAAATTTGATTACGGCGGAAA
GGAATATGAATTCAAAGCCGGAATTTTCGGAATTACTCTCGGAGATCGAGACAGTGAAG
GCAATCGTCCAGCTTACTACTTAGCCGGAAGTGCCATCAAAGTCTACAGCGTCAACACG
AAAGAACTTAAGCAGAAGGGTGGAAAGCTGAATCCGGAGCTTCTTGGAAACCGCGGGA
AGTACAACGATGCCATTGCCCTAGCTTACGATCCCAAAACTAAAGTTATCTTCTTTGCTG
AGGCCAACACAAAGCAAGTATCCTGCTGGAACACACAGAAAATGCCACTGAGGATGAA
GAATACCGACGTAGTCTACACTAGTTCTCGCTTTGTCTTTGGAACGGACATTTCGGTTGA
TAGCAAGGGCGGCCTCTGGTTCATGTCTAACGGCTTTCCGCCTATAAGGAAATCAGAAA
AATTCAAATATGACTTCCCACGCTACCGTCTAATGAGGATCATGGACACACAGGAAGCA
ATTGCCGGAACTGCTTGCGATATGAATGCATAAAAGTTAATTTTCAACCCAAGAAGAAG
ACCTAAAGAGGCTTTTCCAGGCTTTGATGCAGGAGAGGTGGTTATCAACGCAAAATCAG
CTATTGTTGTATGAGGAGGAGAAATTATTGATTCTGAATTCTATAAAAAAAATTTAATTT
GTGAAATATTTGGCAATAATAAATTAATTGAATTACAAAAAAAAAAAAAAAAAAAAAA
AAAAAAA

LJS138 (SEQ ID NO:66)

TCTCTTTGGTTAACATTGTGAAGTTATCGGACGTGGCCGGTTTCTATTTCTTTTGCAAAAA
TGCAGTCAAAAATTCTTTCTTTCGTCCTTTTCACCTTATCCTTGGGCTATGTTTTGGGTGA
AACATGCTCAAATGCTAAGGTTAAGGGAGCTACCTCTTATTCCACAACGGATGCCACAA
TTGTAAGCCAAATTGCCTTTGTGACTGAATTCTCCTTGGAATGCTCAAATCCTGGATCCG
AGAAAATCTCCCTATTTGCTGAAGTCGATGGCAAAATTACTCCTGTTGCCATGATCGGGG
ATACCACCTACCAGGTGAGCTGGAATGAAGAGGTTAATAAGGCTAGAAGTGGTGACTAC
AGTGTGAAGCTGTACGATGAAGAAGGATACGGAGCAGTACGCAAAGCTCAGAGATCAG
GTGAAGAGAACAAGGTCAAACCACTAGCAACCGTTGTTGTTCGACATCCAGGAACATAC
ACTGGACCATGGTTCAATTCCGAAATCCTCGCAGCTGGTCTCATTGCTGTTGTTGCCTAC
TTTGCTTTCTCAACGCGAAGCAAAATTCTTTCCTAAAGAGACGCAGCATGAAATTTCACA
AAAAAATAAAAACAAATTCAAGTCATCAACCATGTCTCTTTGGCACTCAGACTGTTTCTG
TGAAATACAAACTATTATTTAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

LJL124 (SEQ ID NO: 68)

ATTCCCACAAGAAGCTGCTAAAATGGTGTCAATTCTGTTAATCTCCTTGATTCTTAATTT
GTTGGTTTTCTATGCTAAAGCTAGACCACTAGAAGACATCTCGTCAGATCTTTCCCCTGA
TTATTACATCACTGAAGGCTATGACGGTGTGAAGGAGAAGAGAGAGATCGAACTTGTAC
CTGTGACATTTGGAATATTTAATATACATACAACACCTGCTCCCAGAATTACCTTTGAAT
GGTAAAAAATCCAAGAAGAATTTATGATTTTATTCTTCCTTCCATTGGGATGGATTGTAA
GTCAGCATAAAACGCCGTTAAAAATGAATTTTTAATAAAAAAAAATTATTCCAAAAAAA
AAAAAAAAAAAAAAAAAAAAA

LJL35 (SEQ ID NO: 70)

CACTATTCATTGGAAGATTTATTAACTTCAAGATGAAATTATTTTGTTTAATTTTTGTTGT
GTTTGTTGCTTTAGAAGTCTGTATAGAGACCGTGAAAGCTATGGAAGCAACGGAGGAGA
TATCTGTAAAATTGCAAGATGATGCGAATGAACCTGATGACTCTCTGGATTTAGACGAA
GGTCTTCCTGATGCATTCGATGAGGACTATAATAATCAGGCTGAGTACAAGCCGAATCC
TAGAGGGGACTACAGAAGACGATAATTAATATAAATTCAGGAAAACACTCTAAAAATTT
CCAATTGACTCTACTTTAAACGATTTAATACCTACCTACACTAAATACCATATGCAATAA
TTATGTTTTAATTATTTAGTGCAAGATCTACTAGTTTCAGTTCATATTTTGGGACTTTCCC
GCCTTTCTCTCGATGGAAAAATGATTTTACGGATTCTTAATTTTCATTGTACAGAGTTAAT
AAAACAATTGAAAGCAATTAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Also included are fragments of the above-described nucleic acid sequences that are at least 33 bases, at least 36 bases, at least 42 bases or at least 48 bases in length, which is sufficient to permit the fragment to selectively hybridize to a polynucleotide that encodes a disclosed *Lu. longipalpis* under specified conditions. The term "selectively hybridize" refers to hybridization under moderately or highly stringent conditions, which excludes non-related nucleotide sequences.

Also disclosed herein are open reading frames (ORFs) encoding a *Lu. longipalpis* polypeptide. These ORFs are delimited by a start codon and by a stop codon. This also includes the degenerate variants and nucleotide sequences encoding conservative variants and homologs.

Specific, non-limiting examples of open reading frames are as follows:

The LJL34 unprocessed protein is encoded by nucleic acids 30-842 of SEQ ID NO:1, and the mature protein is encoded by the nucleic acid sequence 87-842 of SEQ ID NO:1.

The LJL18 unprocessed protein is encoded by nucleic acids 56-532 of SEQ ID NO:3, and the mature protein is encoded by the nucleic acid sequence 113-532 of SEQ ID NO:3.

The LJS193 unprocessed protein is encoded by nucleic acids 216-502 of SEQ ID NO:5, and the mature protein is encoded by the nucleic acid sequence 276-502 of SEQ ID NO:5.

The LJS201 unprocessed protein is encoded by nucleic acids 48-353 of SEQ ID NO:7, and the mature protein is encoded by the nucleic acid sequence 117-352 of SEQ ID NO:7.

The LJL13 unprocessed protein is encoded by nucleic acids 26-766 of SEQ ID NO:9, and the mature protein is encoded by the nucleic acid sequence 83-766 of SEQ ID NO:9.

The LJL23 unprocessed protein is encoded by nucleic acids 18-992 of SEQ ID NO:11, and the mature protein is encoded by the nucleic acid sequence 81-992 of SEQ ID NO:11.

The LJM10 unprocessed protein is encoded by nucleic acids 92-571 of SEQ ID NO:13, and the mature protein is encoded by the nucleic acid sequence 149-571 of SEQ ID NO:13.

The LJL143 unprocessed protein is encoded by nucleic acids 46-948 of SEQ ID NO:15, and the mature protein is encoded by the nucleic acid sequence 115-948 of SEQ ID NO:15.

The LJS142 unprocessed protein is encoded by nucleic acids 25-507 of SEQ ID NO:17, and the mature protein is encoded by the nucleic acid sequence 85-507 of SEQ ID NO: 17.

The LJL17 unprocessed protein is encoded by nucleic acids 28-342 of SEQ ID NO:19, and the mature protein is encoded by the nucleic acid sequence 88-342 of SEQ ID NO:19.

The LJM06 unprocessed protein is encoded by nucleic acids 50-523 of SEQ ID NO:21, and the mature protein is encoded by the nucleic acid sequence 107-523 of SEQ ID NO:21.

The LJM17 unprocessed protein is encoded by nucleic acids 24-1264 of SEQ ID NO:23, and the mature protein is encoded by the nucleic acid sequence 83-1264 of SEQ ID NO:23.

The LJL04 unprocessed protein is encoded by nucleic acids 30-914 of SEQ ID NO:25, and the mature protein is encoded by the nucleic acid sequence 81-914 of SEQ ID NO:25.

The LJM114 unprocessed protein is encoded by nucleic acids 29-475 of SEQ ID NO:27, and the mature protein is encoded by the nucleic acid sequence 101-475 of NO:27.

The LJM111 unprocessed protein is encoded by nucleic acids 24-1214 of SEQ ID NO:29, and the mature protein is encoded by the nucleic acid sequence 78-1214 of SEQ ID NO:29.

The LJM78mature unprocessed protein is encoded by nucleic acids 42-1091 of SEQ ID NO:31, and the mature protein is encoded by the nucleic acid sequence 102-11091 of SEQ ID NO:31.

The LJS238 unprocessed protein is encoded by nucleic acids 27-206 of SEQ ID NO:33, and the mature protein is encoded by the nucleic acid sequence 87-206 of SEQ ID NO:33.

The LJS169 unprocessed protein is encoded by nucleic acids 11-370 of SEQ ID NO:35, and the mature protein is encoded by the nucleic acid sequence 77-370 of SEQ ID NO:35.

The LJL11 unprocessed protein is encoded by nucleic acids 30-1745 of SEQ ID NO:37, and the mature protein is encoded by the nucleic acid sequence 105-1745 of SEQ ID NO:37.

The LJL08 unprocessed protein is encoded by nucleic acids 26-238 of SEQ ID NO:39, and the mature protein is encoded by the nucleic acid sequence 95-238 of SEQ ID NO:39.

The LJS105 unprocessed protein is encoded by nucleic acids 24-275 of SEQ ID NO:41, and the mature protein is encoded by the nucleic acid sequence 81-275 of SEQ ID NO:41.

The LJL09 unprocessed protein is encoded by nucleic acids 74-1954 of SEQ ID NO:43, and the mature protein is encoded by the nucleic acid sequence 128-1954 of SEQ ID NO:43.

The LJL38 unprocessed protein is encoded by nucleic acids 40-165 of SEQ ID NO:45, and the mature protein is encoded by the nucleic acid sequence 100-165 of SEQ ID NO:45.

The LJM04 unprocessed protein is encoded by nucleic acids 40-456 of SEQ ID NO:47, and the mature protein is encoded by the nucleic acid sequence 100-456 of SEQ ID NO:47.

The LJM26 unprocessed protein is encoded by nucleic acids 96-1616 of SEQ ID NO:49, and the mature protein is encoded by the nucleic acid sequence 147-1616 of SEQ ID NO:49.

The LJS03 unprocessed protein is encoded by nucleic acids 41-553 of SEQ ID NO:51, and the mature protein is encoded by the nucleic acid sequence 98-553 of SEQ ID NO:51.

The LJS192 unprocessed protein is encoded by nucleic acids 18-344 of SEQ ID NO:53, and the mature protein is encoded by the nucleic acid sequence 87-344 of SEQ ID NO:53.

The LJM19 unprocessed protein is encoded by nucleic acids 16-360 of SEQ ID NO:55, and the mature protein is encoded by the nucleic acid sequence 82-360 of SEQ ID NO:55.

The LJL138 unprocessed protein is encoded by nucleic acids 12-1238 of SEQ ID NO:57 and the mature protein is encoded by the nucleic acid sequence 72-1238 of SEQ ID NO:57.

The LJL15 unprocessed protein is encoded by nucleic acids 63-542 of SEQ ID NO:59, and the mature protein is encoded by the nucleic acid sequence 120-542 of SEQ ID NO:59.

The LJL91 unprocessed protein is encoded by nucleic acids 63-542 of SEQ ID NO:61, and the mature protein is encoded by the nucleic acid sequence 120-542 of SEQ ID NO:61.

The LJM11unprocessed protein is encoded by nucleic acids 20-1216 of SEQ ID NO:63, and the mature protein is encoded by the nucleic acid sequence 74-1216 of SEQ ID NO:63.

The LJS138 unprocessed protein is encoded by nucleic acids 12-1238 of SEQ ID NO:65, and the mature protein is encoded by the nucleic acid sequence 72-138 of SEQ ID NO:65.

The LJL124 unprocessed protein is encoded by nucleic acids 23-241 of SEQ ID NO:67, and the mature protein is encoded by the nucleic acid sequence 83-241 of SEQ ID NO:67.

The LJL35 unprocessed protein is encoded by nucleic acids 12-1238 of SEQ ID NO:69, and the mature protein is encoded by the nucleic acid sequence 72-1238 of SEQ ID NO:69.

Another specific non-limiting example of a polynucleotide encoding a *Lu. longipalpis* polypeptide is a polynucleotide having at least 75%, 85%, 90%, 95%, or 99% homology to one of the

sequences set forth above that encodes a polypeptide having an antigenic epitope or function of a *Lu. longipalpis* polypeptide. Yet another specific non-limiting example of a polynucleotide encoding a *Lu. longipalpis* polypeptide is a polynucleotide that encodes a polypeptide that is specifically bound by an antibody that specifically binds the *Lu. longipalpis* polypeptide.

The *Lu. longipalpis* polynucleotides include a recombinant DNA which is incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote, or which exists as a separate molecule (for example, a cDNA) independent of other sequences. The nucleotides can be ribonucleotides, deoxyribonucleotides, or modified forms of either nucleotide. The term includes single and double forms of either nucleotide. The term includes single and double forms of DNA.

Recombinant vectors are also disclosed herein that include a polynucleotide encoding a polypeptide or a fragment thereof according to the disclosure. Recombinant vectors include plasmids and viral vectors and may be used for *in vitro* or *in vivo* expression.

A plasmid may include a DNA transcription unit, for instance a nucleic acid sequence that permit it to replicate in a host cell, such as an origin of replication (prokaryotic or eukaryotic). A plasmid may also include one or more selectable marker genes and other genetic elements known in the art. Circular and linear forms of plasmids are encompassed in the present disclosure.

For *in vivo* expression, the promoter is generally of viral or cellular origin. In one embodiment, the cytomegalovirus (CMV) early promoter (CMV-IE promoter), including the promoter and enhancer, is of use. The CMV-IE promoter can be of human or murine origin, or of other origin such as rat or guinea pig (see EP 0260148; EP 0323597; WO 89/01036; Pasleau *et al.*, *Gene* 38:227-232, 1985; Boshart M. *et al.*, *Cell* 41:521-530, 1985). Functional fragments of the CMV-IE promoter may also be used (WO 98/00166). The SV40 virus early or late promoter and the Rous Sarcoma virus LTR promoter are also of use. Other promoters include but are not limited to, a promoter of a cytoskeleton gene, such as (but not limited to) the desmin promoter (Kwissa M. *et al.*, *Vaccine* 18(22):2337-2344, 2000), or the actin promoter (Miyazaki J. *et al.*, *Gene* 79(2):269-277, 1989). When several genes are present in the same plasmid, they may be provided in the same transcription unit or in different units.

The plasmids may also comprise other transcription regulating elements such as, for example, stabilizing sequences of the intron type. In several embodiments the plasmids include the first intron of CMV-IE (Published PCT Application No. WO 89/01036), the intron II of the rabbit β-globin gene (van Ooyen *et al.*, *Science* 206:337-344, 1979), the signal sequence of the protein encoded by the tissue plasminogen activator (tPA; Montgomery *et al.*, *Cell. Mol. Biol.* 43:285-292, 1997), and/or a polyadenylation signal (polyA), in particular the polyA of the bovine growth hormone (bGH) gene (U.S. Patent No. 5,122,458) or the polyA of the rabbit β-globin gene or of SV40 virus.

In a specific, non-limiting example, the pVR1020 plasmid (VICAL Inc.; Luke C. *et al.*, *Journal of Infectious Diseases* 175:91-97, 1997; Hartikka J. *et al.*, *Human Gene Therapy* 7:1205-

1217, 1996)) can be utilized as a vector for the insertion of such a polynucleotide sequence, generating recombinant plasmids.

The plasmids are evaluated in dogs in order to determine their efficacy against a Leishmania infection (Vidor E. *et al.*, P3.14, XXIV World Veterinary Congress, Rio de Janeiro, Brazil, 18-23 August 1991).

Various viral vectors are also of use with a polynucleotide encoding a *Lu. longipalpis* polypeptide. A specific, non-limiting example includes recombinant poxvirus, including avipox viruses, such as the canarypox virus. Another specific, non-limiting example includes recombinant poxvirus, including vaccinia viruses (U.S. Patent No. 4,603,112), such as attenuated vaccinia virus such as NYVAC (see U.S. Patent No. 5,494,807) or Modified Vaccinia virus Ankara (MVA, Stickl H. and Hochstein-Mintzel V., *Munch. Med. Wschr.* 113:1149-1153, 1971; Sutter G. *et al.*, *Proc. Natl. Acad. Sci. USA* 89:10847-10851, 1992; Carroll M. W. *et al.*, *Vaccine* 15(4):387-394, 1997; Stittelaar K. J. *et al.*, *J. Virol.* 74(9):4236-4243, 2000; Sutter G. *et al.*, *Vaccine* 12(11):1032-1040, 1994). When avipox viruses are used, canarypox viruses (U.S. Patent No. 5,756,103) and fowlpox viruses (U.S. Patent No. 5,766,599) are of use, such as attenuated viruses. For recombinant canarypox virus vectors, the insertion sites may be in particular in the ORFs C3, C5 or C6. When the expression vector is a poxvirus, the heterologous polynucleotide can be inserted under the control of a poxvirus specific promoter, such as the vaccinia virus 7.5kDa promoter (Cochran *et al.*, *J. Virology* 54:30-35, 1985), the vaccinia virus I3L promoter (Riviere *et al.*, *J. Virology* 66:3424-3434, 1992), the vaccinia virus HA promoter (Shida, *Virology* 150:451-457, 1986), the cowpox virus ATI promoter (Funahashi *et al.*, *J. Gen. Virol.* 69:35-47, 1988), other vaccinia virus H6 promoter (Taylor *et al.*, *Vaccine* 6:504-508, 1988; Guo *et al.*, *J. Virol.* 63:4189-4198, 1989; Perkus *et al.*, *J. Virol.* 63:3829-3836, 1989).

Other viral vectors of use are herpes virus or adenovirus vectors. Specific, non-limiting examples include a canine herpes virus (CHV) or canine adenovirus (CAV) vector (for example, see U.S. Patent No. 5,529,780; U.S. Patent No. 5,688,920; Published PCT Application No. WO 95/14102). For CHV, the insertion sites may be in particular in the thymidine kinase gene, in the ORF3, or in the UL43 ORF (see U.S. Patent No. 6,159,477). For CAV, the insertion sites may be in particular in the E3 region or in the region located between the E4 region and the right ITR region (see U.S. Patent No. 6,090,393; U.S. Patent No. 6,156,567). In one embodiment in CHV or CAV vectors the insert is in general under the control of a promoter (as described above for the plasmids), such as CMV-IE promoter.

Multiple insertions can be done in the same vector using different insertion sites or using the same insertion site. When the same insertion site is used, each polynucleotide insert is inserted under the control of different promoters. The insertion can be done tail-to-tail, head-to-head, tail-to-head, or head-to-tail. IRES elements (Internal Ribosome Entry Site, see European Patent EP 0803573) can also be used to separate and to express multiple inserts operably linked to the same promoter. Bacterial vectors can also be used for *in vivo* expression.

Any polynucleotide according to the disclosure can be expressed *in vitro* by DNA transfer or expression vectors into a suitable host cell. The host cell may be prokaryotic or eukaryotic. The term "host cell" also includes any progeny of the subject host cell. Methods of stable transfer, meaning that the foreign polynucleotide is continuously maintained in the host cell, are known in the art. Host cells can include bacteria (for example, *Escherichia coli*), yeast, insect cells, and vertebrate cells. Methods of expressing DNA sequences in eukaryotic cells are well known in the art.

As a method for *in vitro* expression, recombinant Baculovirus vectors (for example, Autographa California Nuclear Polyhedrosis Virus (AcNPV)) can be used with the nucleic acids disclosed herein. For example, polyhedrin promoters can be utilized with insect cells (for example, *Spodoptera frugiperda* cells, like Sf9 cells available at the ATCC under the Accession number CRL-1711, or Sf21 cells) (see for example, Smith *et al.*, *Mol. Cell Biol.* 3:2156-2165, 1983; Pennock *et al.*, *Mol. Cell Biol.* 4: 399-406, 1994; Vialard *et al.*, *J. Virol.* 64:37-50, 1990; Verne A., *Virology* 167:56-71, 1988; O'Reilly *et al.*, "Baculovirus expression vectors, A laboratory manual," New York Oxford, Oxford University Press, 1994; Kidd I. M. & Emery V.C., "The use of baculoviruses as expression vectors," *Applied Biochemistry and Biotechnology* 42:37-159, 1993; European Patent No. EP 0370573; European Patent No. EP 0265785; U.S. Patent No. 4,745,051). For expression the BaculoGold ™ Starter Package (Cat # 21001K) from Pharmingen (Becton Dickinson) can be used.

As a method for *in vitro* expression, recombinant *E. coli* can be used with a vector. For example, when cloning in bacterial systems, inducible promoters such as arabinose promoter, pL of bacteriophage lambda, plac, ptrp, ptac (ptrp-lac hybrid promoter), and the like may be used.

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the $CaCl_2$ method using procedures well known in the art. Alternatively, $MgCl_2$ or RbCl can be used. Transformation can also be performed after forming a protoplast of the host cell if desired, or by electroporation.

When the host is a eukaryote, such methods of transduction of DNA as calcium phosphate coprecipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with *Lu. longipalpis* polynucleotide sequences, and a second foreign DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector (see above), such as a herpes virus or adenovirus (for example, canine adenovirus 2), to transiently transduce eukaryotic cells and express the protein (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982). In addition, a transfection agent can be utilized, such as dioleoyl-phosphatidyl-ethanolamine (DOPE).

Isolation and purification of recombinantly expressed polypeptide may be carried out by conventional means including preparative chromatography (for example, size exclusion, ion exchange, affinity), selective precipitation and ultra-filtration. Such a recombinantly expressed polypeptide is part of the present disclosure. The methods for production of such a polypeptide are

also encompassed, in particular the use of a recombinant expression vector comprising a polynucleotide according to the disclosure and of a host cell.

Antibodies

A *Lu. longipalpis* polypeptide of the disclosure or a fragment thereof according to the disclosure can be used to produce antibodies. Polyclonal antibodies, antibodies which consist essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct monoclonal antibodies are included. Such antibodies are of use as markers for exposure, and as immunodiagnostic tools to follow the development of the immune response to *Lu. longipalpis* salivary proteins.

The preparation of polyclonal antibodies is well-known to those skilled in the art. See, for example, Green et al., "Production of Polyclonal Antisera," *Immunochemical Protocols*, pp. 1-5, Manson, ed., *Humana Press,* 1992; Coligan *et al.*, "Production of Polyclonal Antisera in Rabbits, Rats, Mice and Hamsters," *Current Protocols in Immunology*, section 2.4.1, 1992.

The preparation of monoclonal antibodies likewise is conventional. See, for example, Kohler & Milstein, *Nature* 256:495, 1975; Coligan *et al.*, sections 2.5.1-2.6.7; and Harlow *et al.*, *Antibodies: A Laboratory Manual*, p. 726, Cold Spring Harbor Pub., 1988. Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising an antigen, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B lymphocytes, fusing the B lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures. Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, for example, Coligan *et al.*, sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes *et al.*, "Purification of Immunoglobulin G (IgG)," *Methods in Molecular Biology*, Vol. 10, pp. 79-104, Humana Press, 1992.

Methods of *in vitro* and *in vivo* multiplication of monoclonal antibodies are well known to those skilled in the art. Multiplication *in vitro* may be carried out in suitable culture media such as Dulbecco's Modified Eagle Medium or RPMI 1640 medium, optionally supplemented by a mammalian serum such as fetal calf serum or trace elements and growth-sustaining supplements such as normal mouse peritoneal exudate cells, spleen cells, thymocytes, or bone marrow macrophages. Production *in vitro* provides relatively pure antibody preparations and allows scale-up to yield large amounts of the desired antibodies. Large-scale hybridoma cultivation can be carried out by homogenous suspension culture in an airlift reactor, in a continuous stirrer reactor, or in immobilized or entrapped cell culture. Multiplication *in vivo* may be carried out by injecting cell clones into mammals histocompatible with the parent cells, for example, syngeneic mice, to cause growth of antibody-producing tumors. Optionally, the animals are primed with a hydrocarbon, especially oils

such as pristane (tetramethylpentadecane) prior to injection. After one to three weeks, the desired monoclonal antibody is recovered from the body fluid of the animal.

Antibodies can also be derived from subhuman primate antibody. General techniques for raising therapeutically useful antibodies in baboons can be found, for example, in WO 91/11465, 1991, and Losman *et al.*, *Int. J. Cancer* 46:310, 1990.

Alternatively, an antibody that specifically binds a polypeptide can be derived from a humanized monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementarity determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain, and then substituting human residues in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:3833, 1989. Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321:522, 1986; Riechmann *et al.*, *Nature* 332:323, 1988; Verhoeyen *et al.*, *Science* 239:1534, 1988; Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89:4285, 1992; Sandhu, *Crit. Rev. Biotech.* 12:437, 1992; and Singer *et al.*, *J. Immunol.* 150:2844, 1993.

Antibodies can be derived from human antibody fragments isolated from a combinatorial immunoglobulin library. See, for example, Barbas *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, p. 119, 1991; Winter *et al.*, *Ann. Rev. Immunol.* 12:433, 1994. Cloning and expression vectors that are useful for producing a human immunoglobulin phage library can be obtained, for example, from STRATAGENE Cloning Systems (La Jolla, CA).

In addition, antibodies can be derived from a human monoclonal antibody. Such antibodies are obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain loci are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green *et al.*, *Nature Genet.* 7:13, 1994; Lonberg *et al.*, *Nature* 368:856, 1994; and Taylor *et al.*, *Int. Immunol.* 6:579, 1994.

Antibodies include intact molecules as well as fragments thereof, such as Fab, $F(ab')_2$, and Fv which are capable of binding the epitopic determinant. These antibody fragments retain some ability to selectively bind with their antigen or receptor and are defined as follows:

(1) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain (L) and a portion of one heavy chain(H);

(2) Fab', the fragment of an antibody molecule that can be obtained by treating a whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule;

(3) $(Fab')_2$, the fragment of the antibody that can be obtained by treating a whole antibody with the enzyme pepsin without subsequent reduction; $F(ab')_2$ is a dimer of two Fab' fragments held together by two disulfide bonds;

(4) Fv, defined as a genetically engineered fragment containing the variable region of the light chain (V_L) and the variable region of the heavy chain (V_H) expressed as two chains; and

(5) Single chain antibody (SCA), defined as a genetically engineered molecule containing the variable region of the light chain, the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

Methods of making these fragments are known in the art. (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual,* Cold Spring Harbor Laboratory, New York, 1988).

Antibody fragments can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly (see U.S. Patent No. 4,036,945 and U.S. Patent No. 4,331,647, and references contained therein; Nisonhoff *et al., Arch. Biochem. Biophys.* 89:230, 1960; Porter, *Biochem. J.* 73:119, 1959; Edelman *et al., Methods in Enzymology,* Vol. 1, page 422, Academic Press, 1967; and Coligan *et al.* at sections 2.8.1-2.8.10 and 2.10.1-2.10.4).

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent (Inbar *et al., Proc. Nat'l Acad. Sci. USA* 69:2659, 1972). Alternatively, the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. See, for example, Sandhu, *supra.* In one embodiment, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli.* The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are known in the art (see Whitlow *et al., Methods: a Companion to Methods in*

Enzymology, Vol. 2, page 97, 1991; Bird *et al.*, *Science* 242:423, 1988; U.S. Patent No. 4,946,778; Pack *et al.*, *Bio/Technology* 11:1271, 1993; and Sandhu, *supra)*.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (Larrick *et al.*, *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 106, 1991).

Antibodies can be prepared using an intact polypeptide or fragments containing small peptides of interest as the immunizing antigen. The polypeptide or a peptide used to immunize an animal can be derived from substantially purified polypeptide produced in host cells, *in vitro* translated cDNA, or chemical synthesis which can be conjugated to a carrier protein, if desired. Such commonly used carriers which are chemically coupled to the peptide include keyhole limpet hemocyanin (KLH), thyroglobulin, bovine serum albumin (BSA), and tetanus toxoid. The coupled peptide is then used to immunize an animal (for example, a mouse, a rat, or a rabbit).

Polyclonal or monoclonal antibodies can be further purified, for example, by binding to and elution from a matrix to which the polypeptide or a peptide to which the antibodies were raised is bound. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (See for example, Coligan *et al.*, Unit 9, *Current Protocols in Immunology*, Wiley Interscience, 1991).

It is also possible to use the anti-idiotype technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region that is the "image" of the epitope bound by the first mono-clonal antibody.

In view of the large number of methods that have been reported for attaching a variety of radiodiagnostic compounds, radiotherapeutic compounds, label (for example, enzymes or fluorescent molecules) drugs, toxins, and other agents to antibodies one skilled in the art will be able to determine a suitable method for attaching a given agent to an antibody or other polypeptide.

In one embodiment, an antibody that binds a *Lu. Longipalpis* polypeptide can be used to assess whether a subject has been bitten by a sand fly. In one specific, non-limiting example, a sample is obtained from a subject of interest, such as a human or a dog. The sample can be a body fluid (for example, blood, serum, urine, saliva, etc.) or a tissue biopsy. The sample or a fraction thereof is contacted with the antibody, and the ability of the antibody to form an antigen-antibody complex is assessed. One of skill in the art can readily detect the formation of an antigen-antibody complex. For example, ELISA, Western blot, or radio-immune assays can be utilized.

Immunogenic Compositions, Vaccines and Methods of Use

Immunogenic compositions and vaccines are disclosed herein. In one embodiment the immunogenic compositions and vaccines include a polypeptide. In another embodiment, the

immunogenic compositions and vaccines include a recombinant vector, such as a viral vector or a plasmid. When administered to a subject such an immunogenic composition or vaccine generates an immune response to the sand fly's salivary protein(s), and surprisingly a reduction of the leishmaniasis symptoms and a decrease of the leishmania parasite load results. Thus, without being bound by theory, a cellular response, such as a Th1 response, produced against the salivary protein can indirectly kill a Leishmania parasite. For example, a Th1 type response can allow macrophages to take up Leishmania antigens and present them to T cells in a Th1 context. The induction the Th1 response can produce an anti-Leishmania immune response, or can prime the immune system of the mammalian host for anti-Leishmania immunity in response to a later infection.

In one embodiment, the immunogenic composition or the vaccine includes an effective amount of at least one *Lu. longipalpis* polypeptide disclosed herein. The immunogenic composition and the vaccine can include a pharmaceutically acceptable excipient and/or an adjuvant. In one embodiment, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. In one specific, non-limiting example, the immunogenic composition or vaccine includes a polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67. In specific, non-limiting examples, the immunogenic composition includes a polypeptide having a sequence set forth as one of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59.

In one embodiment, the immunogenic composition includes more than one *Lu. longipalpis* polypeptide, such as two, three, four, five, six, ten or more of the polypeptides disclosed herein. Thus, the immunogenic composition includes at least one polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these

polypeptides, a conservative variant, a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, and optionally another polypeptide having an amino acid sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or SEQ ID NO: 69, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant of one of these polypeptides, or a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides.

In specific non-limiting examples, the immunogenic composition includes an amino acid having a sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, SEQ ID NO: 39, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant of one of these polypeptides, or a homolog or an immunogenic fragment comprising at least eight or at least ten consecutive amino acids of one of these polypeptides, or a combination of these polypeptides. Thus, the immunogenic composition can include a polypeptide having a sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39. These compositions include, but are not limited to, an immunogenic composition including a polypeptide having a sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59, and a polypeptide having a sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39.

The immunogenic composition or the vaccine can include a pharmaceutically acceptable excipient and/or an adjuvant.

In another embodiment, the immunogenic composition or the vaccine includes an effective amount of at least one *Lu. longipalpis* polypeptide in conjunction with one or more *P. perniciosus* polypeptide(s) and/or one or more *P. ariasi* polyeptide(s). These polypeptide sequences are disclosed in U.S. Patent Application No. 60/412,327, filed September 19, 2002, U.S. Patent Application No. 60/425,852, filed November 12, 2002, and PCT Application No. PCT/US03/29833, filed September 18, 2003, which are incorporated herein by reference.

In one embodiment, the immunogenic composition or the vaccine comprises an effective amount of a recombinant vector expressing at least one *Lu. longipalpis* polypeptide disclosed herein and a pharmaceutically acceptable vehicle or excipient. In one specific, non-limiting example the recombinant vector encodes at least one polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ

ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a conservative variant, a homolog, an immunogenic fragment, or a fusion protein thereof. In specific non-limiting examples the vector encodes a polypeptide having a sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59, a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant, a homolog, an immunogenic fragment, or a fusion protein thereof. In several examples the vector encodes one or more polypeptides having a sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59. The vector can also optionally encode a polypeptide having a sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39.

The immunogenic composition can include a nucleic acid sequence encoding a *P. ariasi* polypeptide(s) and/or a *P. perniciosus* polypeptide(s) (see U.S. Provisional Application No. 60/412,327, which is incorporated by reference herein in its entirety). In one embodiment, the *Lu. longipalpis* polypeptide(s) having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, a conservative variant, a homolog, an immunogenic fragment, or a fusion protein thereof, are encoded by the same recombinant vector as a *P. ariasi* polypeptide(s) and/or a *P. perniciosus* polypeptide(s). In another embodiment, the *Lu. longipalpis* polypeptide(s), a *P. ariasi* polypeptide(s) and/or a *P. perniciosus* polypeptide(s), are encoded by different recombinant vectors.

The *Lu. longipalpis* polypeptide can be administered by any means known to one of skill in the art (See Banga, A., "Parenteral Controlled Delivery of Therapeutic Peptides and Proteins," *Therapeutic Peptides and Proteins*, Technomic Publishing Co., Inc., Lancaster, PA, 1995) such as by intramuscular (IM), intradermal (ID), subcutaneous (SC), or intravenous injection, but even oral, nasal, or anal administration is contemplated. In one embodiment, administration is by subcutaneous, intradermal, or intramuscular injection using a needleless injector (Biojector™, Bioject, Oregon, USA).

To extend the time during which the peptide or protein is available to stimulate a response, the peptide or protein can be provided as an implant, an oily injection, or as a particulate system. The particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar particle. (see, for example, Banja, *supra)*. A particulate carrier based on a synthetic polymer has been shown to act as an adjuvant to enhance the immune response, in addition to providing a controlled release. Aluminum salts may also be used as adjuvants to produce a humoral immune response. Thus, in one embodiment, a *Lu. longipalpis* polypeptide is administered in a manner to induce a humoral response.

In another embodiment, a *Lu. longipalpis* polypeptide is administered in a manner to direct the immune response to a cellular response (that is, a CTL response), rather than a humoral (antibody) response. A number of means for inducing cellular responses, both *in vitro* and *in vivo*, are known. Lipids have been identified as agents capable of assisting in priming CTL *in vivo* against various antigens. For example, as described in U.S. Patent No. 5,662,907, palmitic acid residues can be attached to the alpha and epsilon amino groups of a lysine residue and then linked (for example, via one or more linking residues, such as glycine, glycine-glycine, serine, serine-serine, or the like) to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated in a liposome, or emulsified in an adjuvant. As another example, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylseryl-serine can be used to prime tumor specific CTL when covalently attached to an appropriate peptide (see, Deres *et al.*, *Nature* 342:561, 1989). Further, as the induction of neutralizing antibodies can also be primed with the same molecule conjugated to a peptide which displays an appropriate epitope, the two compositions can be combined to elicit both humoral and cell-mediated responses where that is deemed desirable.

In yet another embodiment, an MHC class II-restricted T-helper epitope is added to the polypeptide of the disclosure to induce T-helper cells to secrete cytokines in the microenvironment to activate CTL precursor cells. The technique further involves adding short lipid molecules to retain the construct at the site of the injection for several days to localize the antigen at the site of the injection and enhance its proximity to dendritic cells or other "professional" antigen presenting cells over a period of time (see Chesnut *et al.*, "Design and Testing of Peptide-Based Cytotoxic T-Cell-Mediated Immunotherapeutics to Treat Infectious Diseases and Cancer," Powell, *et al.*, (eds.), *Vaccine Design, the Subunit and Adjuvant Approach*, Plenum Press, New York, 1995).

An immunogenic composition or a vaccine according to the disclosure can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species, and condition of the particular subject, and the route of administration. The immunogenic composition or the vaccine can be administered alone, or in combination with adjuvant(s) and/or with other antigen(s). The other antigen(s) can be a Leishmania antigen. In one embodiment, the Leishmania antigen is the A2 antigen, such as the A2 antigen from *L. infantum* (see Published PCT Patent Application No. WO 95/06729 and in particular the sequence given in SEQ ID NO:2). The other antigen(s) can be present in the composition as a protein, or as an immunological fragment thereof (for example, an epitope), or as an insert in an expression vector (for example, recombinant viral vector, recombinant plasmid, in particular the pVR1012 (Vical Inc.; Hartikka J. *et al.*, *Human Gene Therapy* 7:1205-1217, 1996)).

Any immunogenic composition, vaccine, or therapeutic composition according to the disclosure can be mixed with an adjuvant.

Polypeptide-based compositions:

In several embodiments, the polypeptide-based immunogenic compositions and vaccines according to the disclosure are formulated with (1) vitamin E, saponin (for example, Quil A™, QS21™), aluminum hydroxide, aluminum phosphate, aluminum oxide ("Vaccine Design, The subunit and adjuvant approach," *Pharmaceutical Biotechnology*, vol. 6, Edited by Micheal F. Powell and Mark J. Newman, 1995, Plenum Press New York), (2) an acrylic acid or methacrylic acid polymer, a polymer of maleic anhydride and of alkenyl derivative, (3) an immunostimulating sequence (ISS), in particular an oligodeoxyribonucleotidic sequence bearing one or more non-methylated CpG groups (Klinman D. M. *et al.*, *Proc. Natl. Acad. Sci. USA* 93:2879-2883, 1996; Published PCT Application No. WO 98/16247), (4) to formulate the immunogenic or vaccine preparation in the form of an oil-in-water emulsion, in particular the SPT emulsion described on page 147 of "Vaccine Design, The Subunit and Adjuvant Approach" edited by M. Powell and M. Newman, Plenum Press, 1995, and the emulsion MF59 described on page 183 of this same book, (5) cytokines, or (6) combinations or mixtures thereof.

The cytokines (5) that can be added to the composition, include, but are not limited to, GM-CSF (granulocyte-macrophage colony stimulating factor) or cytokines inducing Th1 (for example, IL-12). All these cytokines can be added to the composition as a protein or as a vector encoding this cytokine protein. In one embodiment, the cytokines are from canine origin, for example, canine GM-CSF, for which a gene sequence has been deposited at the GenBank database (Accession No. S49738). This sequence can be used to create the vector in a manner similar to what was made in the Published PCT Patent Application No. WO 00/77210.

In one specific, non-limiting example the adjuvant contains two or more of an emulsifier, a micelle-forming agent, and an oil. Suitable emulsifiers, micelle-forming agents, and oils are detailed in U.S. Patent Nos. 5, 585,103; 5,709,860; 5,270,202; and 5,695,770, all of which are incorporated by reference. An emulsifier is any molecule that allows the components of the emulsion to remain as a stable emulsion. Such emulsifiers include polysorbate 80 (Sorbitan-mono-9-octadecenoate-poly(oxy-1,2-ethanediyl); manufactured by ICI Americas, Wilmington, Del.), polysorbate 20, polysorbate 21, polysorbate 40, polysorbate 60, polysorbate 61, polysorbate 85, dodecyl-N,N-dimethyl-3-ammonio-1-propanesulfonate, TEEPOL HB7 ™, and SPAN 80 ™ SPAN 85 ™, ethoxylated fatty alcohols, ethoxylated fatty acids, ethoxylated castor oil (hydrogenated or not). In one embodiment, these emulsifiers are provided in an amount of approximately 0.05 to approximately 0.5%. In another embodiment, these emulsifiers are provided in an amount of approximately 0.2%. A micelle forming agent is an agent which is able to stabilize the emulsion formed with the other components such that a micelle-like structure is formed.

Examples of such agents include polymer surfactants described by BASF Wyandotte publications, for example, Schmolka, *J. Am. Oil. Chem. Soc.* 54:110, 1977, and Hunter *et al.*, *J. Immunol.* 129:1244, 1981, PLURONIC™ L62LF, L101, L121, and L64, PEG1000, and TETRONIC™ 1501, 150R1, 701, 901, 1301, and 130R1. The chemical structures of such agents are well known in the art. In one embodiment, the agent is chosen to have a hydrophile-lipophile balance

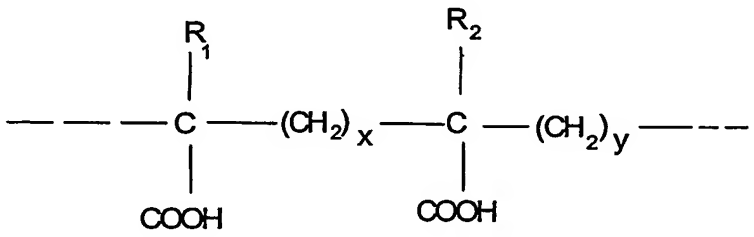
(HLB) of between about 0 and about 2, as defined by Hunter and Bennett, *J. Immun.* 133:3167, 1984. In one embodiment, the agent can be provided in an effective amount, for example between about 0.5 and about 10%. In another embodiment, the agent can be provided in an effective amount, for example between about 1.25 and about 5%.

In one embodiment, the oil included in the composition is chosen to promote the retention of the antigen in oil-in-water emulsion, for instance, to provide a vehicle for the desired antigen. In another embodiment, the oil has a melting temperature of less than about 65° C such that emulsion is formed either at room temperature (about 20° C to about 25° C), or once the temperature of the emulsion is brought down to room temperature.

The oil-in-water emulsion (4) can be based in particular on light liquid paraffin oil (European Pharmacopea type); isoprenoid oil such as squalane, squalene, EICOSANE™ or tetratetracontane; oil resulting from the oligomerization of alkenes, in particular of isobutene or decene; esters of acids or of alcohols containing a linear alkyl group, more particularly plant oils, ethyl oleate, propylene glycol di(caprylate/caprate), glyceryl tri(caprylate/caprate) or propylene glycol dioleate; esters of branched fatty acids or alcohols, in particular isostearic acid esters. The oil is used in combination with emulsifiers to form the emulsion. In several embodiments, the emulsifiers are nonionic surfactants, in particular esters of sorbitan, mannide (for example, anhydromannitol oleate), glycerol, polyglycerol, propylene glycol, and oleic, isostearic, ricinoleic, or hydroxystearic acid, which are optionally ethoxylated, and polyoxypropylene-polyoxyethylene copolymer blocks, in particular the Pluronic® products, especially L121. In one specific, non-limiting example, the oil is provided in an amount between about 1 and about 60%. In another specific, non-limiting example, the oil is provided in an amount between about 5 and about 30%. In one embodiment, the adjuvant is a mixture of emulsifiers, micelle-forming agent, and oil available under the name Provax® (IDEC Pharmaceuticals, San Diego, CA).

The acrylic acid or methacrylic acid polymers (2) can be cross-linked in particular with polyalkenyl ethers of sugars or of polyalcohols. These compounds are known under the term "carbomer" (*Pharmeuropa*, Vol. 8, No. 2, June 1996). A person skilled in the art may also refer to U.S. Patent No. 2,909,462 (incorporated by reference) describing such acrylic polymers cross-linked with a polyhydroxylated compound containing at least 3 hydroxyl groups. In one embodiment, a polyhydroxylated compound contains not more than 8 hydroxyl groups. In another embodiment, the hydrogen atoms of at least 3 hydroxyls are replaced with unsaturated aliphatic radicals containing at least 2 carbon atoms. In other embodiments, radicals contain from about 2 to about 4 carbon atoms, for example, vinyls, allyls, and other ethylenically unsaturated groups. The unsaturated radicals can themselves contain other substituents, such as methyl. The products sold under the name Carbopol® (Noveon Inc., Ohio, USA) are particularly suitable. They are cross-linked with an allyl sucrose or with allylpentaerythritol. Among these, mention may be made of the products Carbopol® 974P, 934P, and 971P.

The copolymers of maleic anhydride and of an alkenyl derivative, such as the EMA® products (Monsanto) which are copolymers of maleic anhydride and of ethylene, may be linear or cross-linked, for example cross-linked with divinyl ether. Reference may be made to J. Fields *et al.*, *Nature* 186:778-780, 1960 (incorporated by reference). In one embodiment, the acrylic acid or methacrylic acid polymers and the EMA® products are formed from units based on the following formula:



in which:

- R_1 and R_2, which may be identical or different, represent H or CH_3
- $x = 0$ or 1, in one embodiment, $x = 1$
- $y = 1$ or 2, with $x + y = 2$.

For the EMA® products, $x = 0$ and $y = 2$. For the carbomers, $x = y = 1$.

In one embodiment, the dissolution of these polymers in water leads to an acid solution, which is neutralized to physiological pH, in order to give to the subject the adjuvant solution into which the immunogenic composition or the vaccine itself is incorporated. The carboxyl groups of the polymer are then partly in COO^- form.

In one embodiment, a solution of adjuvant, especially of carbomer, is prepared in distilled water. In another embodiment, a solution of adjuvant, especially of carbomer, is prepared in the presence of sodium chloride, the solution obtained being at acidic pH. In another embodiment, this stock solution is diluted by adding it to the desired quantity (for obtaining the desired final concentration), or a substantial part thereof, of water charged with NaCl. In yet another embodiment, stock solution is diluted by adding it to the desired quantity of physiological saline (NaCl 9 g/l) all at once in several portions with concomitant or subsequent neutralization (pH 7.3 to 7.4). In one embodiment, the stock solution is neutralized with NaOH. This solution at physiological pH is used as it is for mixing with the immunogenic composition or with the vaccine, which may be especially stored in freeze-dried, liquid or frozen form.

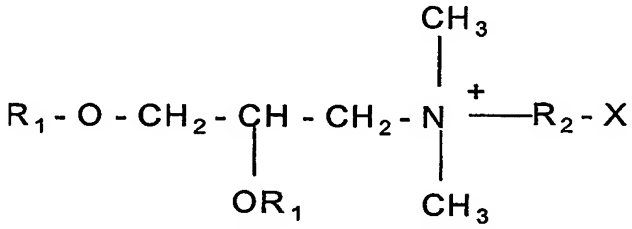
In one embodiment, the polymer concentration in the final vaccine composition is from about 0.01 to about 1.5% W/V. In another embodiment, the final vaccine composition is from about 0.05 to about 1% W/V. In yet another embodiment, the final vaccine composition is from about 0.1 to about 0.4% W/V.

Lipids have been identified as agents capable of stimulating the immune response for various antigens. For example, as described in U.S. Patent No. 5,662,907, palmitic acid residues can be attached to the alpha and epsilon amino groups of a lysine residue and then linked (for example, via one or more linking residues, such as glycine, glycine-glycine, serine, serine-serine, or the like) to an immunogenic peptide. The lipidated peptide can then be injected directly in a micellar form, incorporated in a liposome, or emulsified in an adjuvant. As another example, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylseryl-serine, can be used.

To extend the time during which the peptide or protein is available to stimulate a response, the peptide or protein can be provided as an implant, an oily injection, or as a particulate system. The particulate system can be a microparticle, a microcapsule, a microsphere, a nanocapsule, or similar particle. (see, for example, Banja, *supra)*. A particulate excipient based on a synthetic polymer has been shown to act as an adjuvant to enhance the immune response, in addition to providing a controlled release.

Plasmid-based compositions:

In one embodiment, the plasmid-based compositions is formulated with cationic lipids, in particular with cationic lipids containing a quaternary ammonium salt having the following formula:



in which R1 is a saturated or unsaturated linear aliphatic radical from 12 to 18 carbon atoms, R2 is another aliphatic radical comprising from 2 to 3 carbon atoms, and X is an hydroxyl or amine group.

In one embodiment, DMRIE (N-(2-hydroxyethyl)-N,N-dimethyl-2,3-bis(tetradecyloxy)-1-propanammonium; Published PCT Application No. WO 96/34109) is the cationic lipid. In another embodiment, the cationic lipid is in association with a neutral lipid, for example DOPE (dioleoyl-phosphatidyl-ethanolamine; Behr J. P., *Bioconjugate Chemistry* 5:382-389, 1994), in order to form the DMRIE-DOPE. In yet another embodiment, the mixture is made extemporaneously about 10 minutes to about 60 minutes before administration. In another embodiment, the mixture is made extemporaneously about 30 minutes before administration. In one embodiment, the molar ratio DMRIE/DOPE is from about 95/5 to about 5/95. In another embodiment, the molar ratio DMRIE/DOPE is about 1/1. In one embodiment, the weight ratio plasmid/DMRIE or DMRIE-DOPE adjuvant is from about 50/1 to about 1/10. In another embodiment, the weight ratio plasmid/DMRIE or DMRIE-DOPE adjuvant is from about 10/1 to about 1/5. In yet another embodiment, the weight ratio plasmid/DMRIE or DMRIE-DOPE adjuvant is from about 1/1 to about 1/2.

In one embodiment, a cytokine or non-methylated CpG groups is added to the composition, as described above for polypeptide-based compositions. The addition can be done advantageously by a plasmid encoding the cytokine.

Viral vector-based composition:

The recombinant viral vector-based composition can be supplemented with fMLP (N-formyl-methionyl-leucyl-phenylalanine; U.S. Patent No. 6,017,537) and/or acrylic acid or methacrylic acid polymer adjuvant as described above for polypeptide-based compositions. They can also be formulated with emulsions as described above.

In one embodiment, cytokines, non-methylated CpG groups, or emulsions are added to the composition as described above for polypeptide-based compositions. The addition can be done advantageously by a viral vector encoding said cytokine.

The immunogenic compositions and vaccines according to the disclosure are conserved and stored either in formulated form at 5°C, or in lyophilized form. In one embodiment, the immunogenic compositions and vaccines according to the disclosure are conserved and stored either in formulated form at 5°C, or in lyophilized form with a stabilizer. Freeze-drying can be done according to well-known standard freeze-drying procedures. The pharmaceutically acceptable stabilizers may be SPGA (sucrose phosphate glutamate albumin) (Bovarnik *et al., J. Bacteriology* 59:509, 1950), carbohydrates (for example, sorbitol, mannitol, lactose, sucrose, glucose, dextran, trehalose), sodium glutamate (Tsvetkov T. *et al., Cryobiology* 20(3):318-23, 1983 ; Israeli E. *et al., Cryobiology* 30(5):519-23, 1993), proteins such as peptone, albumin, or casein, protein containing agents such as skimmed milk (Mills CK *et al., Cryobiology* 25(2):148-52, 1988; Wolff E. *et al., Cryobiology* 27(5):569-75, 1990), and buffers (for example, phosphate buffer, alkaline metal phosphate buffer). An adjuvant may be used to make soluble the freeze-dried preparations.

Methods of Immunization

The present disclosure provides methods for inducing an immune response to a Lutzomyia sand fly polypeptide in a subject. The present disclosure provides further methods for inhibiting or preventing leishmaniasis in a subject.

These methods include the administration of at least one immunogenic composition or vaccine according to the disclosure.

An immunogenic composition or a vaccine according to the disclosure can be prepared in accordance with standard techniques well known to those skilled in the pharmaceutical or veterinary art. Such compositions can be administered in dosages and by techniques well known to those skilled in the medical or veterinary arts, taking into consideration such factors as the age, sex, weight, species, and condition of the particular subject, and the route of administration.

If more than one administration is required, they can be administered concurrently (for example, different compositions given during the same period of time via the same or different routes, or a same composition given in the same period of time via different routes), or sequentially

(for example, the same or different compositions given at least two times via the same or different routes). In one embodiment, the delay between two sequential administrations is from about 1 week to about 6 months. In another embodiment, the delay is from about 3 weeks to about 6 weeks. In yet another embodiment, the delay is from about 4 weeks. Following vaccination, annual boost administrations may be done. Advantageously, in a prime-boost vaccination schedule, at least one prime-administration can be done with a composition containing a plasmid according to the disclosure, following by at least one booster administration done with a composition containing a recombinant viral vector according to the disclosure, on the condition that a same *Lu. longipalpis* salivary polypeptide is present twice, coded by the plasmid and by the viral vector. Alternatively, the booster administration can be done with a composition containing a polypeptide according to the disclosure, on the condition that a same *Lu. longipalpis* salivary polypeptide is present twice, coded by the prime-administration plasmid and in the booster polypeptide-based composition.

In such compositions the antigen(s) may be in admixture with a suitable vehicle or excipient such as sterile water, physiological saline, glucose, or the like. The compositions can contain auxiliary substances such as wetting or emulsifying agents, pH buffering agents, adjuvants, gelling, or viscosity enhancing additives, preservatives, flavoring agents, colors, and the like, depending upon the route of administration and the preparation desired. Standard texts, such as Remington's Pharmaceutical Science, 17th edition, 1985, incorporated herein by reference, may be consulted to prepare suitable preparations, without undue experimentation. The compositions can also be lyophilized.

Suitable dosages can also be based upon the examples below. For polypeptide-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector™ (Bioject, Oregon, USA). In several embodiments, polypeptide dosages can be from about 1 to 250 μg/ml, from about 15 to about 150 μg/dose, or from about 20 to about 100 μg/dose. In another embodiment, using a needle-less apparatus the volume of a dose can be between about 0.1 ml and about 0.5 ml. In yet another embodiment, using a needle-less apparatus the volume of a dose can be about 0.25 ml. Administration with multiple points of injection is preferred. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2 ml. In another embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.5 to about 1 ml.

For plasmid-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector™. The dosage is from about 50 μg to about 500 μg per plasmid. When DMRIE-DOPE is added, about 100 μg per plasmid is preferred. In one embodiment, when canine GM-CSF or other cytokine is used, the plasmid encoding this protein is present at a dosage from about 200 μg to about 500 μg. In another embodiment, the plasmid encoding this protein is present at a dosage of about 200 μg. In one embodiment, using a needle-less apparatus, the volume of a dose can be between about 0.1 ml and about 0.5 ml. In another embodiment, the volume

of a dose can be about 0.25 ml. In yet another embodiment, administration is performed using multiple points of injection. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2 ml. In another embodiment, the volumes are from about 0.5 to about 1 ml. The dosage are the same than mentioned above.

For recombinant viral vector-based compositions, the route of administration can be ID, IM, SC, intravenous, oral, nasal, or anal. This administration can be made with a syringe and a needle or with a needle-less apparatus like, for example, Biojector™. The dosage is from about 10^3 pfu to about 10^9 pfu per recombinant poxvirus vector. In one embodiment, when the vector is a canarypox virus, the dosage is from about 10^5 pfu to about 10^9 pfu. In another embodiment, the dosage is from about 10^6 pfu to about 10^8 pfu. In one embodiment, the volume of needle-less apparatus doses could be between about 0.1 ml and about 0.5 ml. In another embodiment, the volume of needle-less apparatus dose is 0.25 ml. In yet another embodiment, administration is performed using multiple points of injection. In one embodiment, for conventional injection with a syringe and a needle, the volumes are from about 0.1 to about 2 ml. In another embodiment, the volumes are from about 0.5 to about 1 ml. The dosages are the same as mentioned above. In one embodiment, when a syringe with a needle is used, the injection is IM.

Advantageously for the prime boost administration regimen, the prime-administration is made with a plasmid-based composition and the boost administration is made with a recombinant viral vector-based composition. In one embodiment, the boost administration is made with a canarypox vector. Both priming and boosting administrations include vectors encoding at least one identical *Lu. longipalpis* salivary antigens, and optionally Leishmania A2 antigens. The dosage of plasmids and recombinant viral vectors are the same as above. Optionally, the boost administration can be done with a polypeptide-based composition. In this case, the dosage of polypeptide is from about 1 to about 250 μg/ml, from about 15 to about 150 μg/dose, or from about 20 to about 100 μg/dose.

Immunization by nucleic acid constructs is well known in the art and taught, for example, in U.S. Patent No. 5,643,578 (which describes methods of immunizing vertebrates by introducing DNA encoding a desired antigen to elicit a cell-mediated or a humoral response) and U.S. Patent No. 5,593,972 and U.S. Patent No. 5,817,637 (which describe operably linking a nucleic acid sequence encoding an antigen to regulatory sequences enabling expression). U.S. Patent No. 5,880,103 describes several methods of delivery of nucleic acids encoding immunogenic peptides or other antigens to an organism. The methods include liposomal delivery of the nucleic acids (or of the synthetic peptides themselves), and immune-stimulating constructs, or ISCOMS™, negatively charged cage-like structures of 30-40 nm in size formed spontaneously on mixing cholesterol and Quil A™ (saponin). Protective immunity has been generated in a variety of experimental models of infection, including toxoplasmosis and Epstein-Barr virus-induced tumors, using ISCOMS™ as the delivery vehicle for antigens (Mowat and Donachie, *Immunol. Today* 12:383, 1991). Doses of antigen as low as 1 μg encapsulated in ISCOMS™ have been found to produce class I mediated CTL responses (Takahashi *et al.*, *Nature* 344:873, 1990).

In another approach to using nucleic acids for immunization, a *Lu. longipalpis* polypeptide, or an immunogenic fragment thereof, can also be expressed by attenuated viral hosts or vectors or bacterial vectors. Recombinant vaccinia virus, adeno-associated virus (AAV), herpes virus, retrovirus, or other viral vectors can be used to express the peptide or protein, thereby eliciting a CTL response. For example, vaccinia vectors and methods useful in immunization protocols are described in U.S. Patent No. 4,722,848. BCG (Bacillus Calmette Guerin) provides another vector for expression of the peptides (see Stover, *Nature* 351:456-460, 1991).

In one embodiment, a nucleic acid encoding a *Lu. longipalpis* polypeptide, or an immunogenic fragment thereof, is introduced directly into cells. For example, the nucleic acid may be loaded onto gold microspheres by standard methods and introduced into the skin by a device such as Bio-Rad's Helios™ Gene Gun. A needless injector can also be utilized, such as a Bioinjector2000™. The nucleic acids can be "naked," consisting of plasmids under control of a strong promoter. Typically, the DNA is injected into muscle, although it can also be injected directly into other sites. Exemplary dosages for injection are around 0.5 μg/kg to about 50 mg/kg, and typically are about 0.005 mg/kg to about 5 mg/kg (see, for example, U.S. Patent No. 5,589,466). In one embodiment, a prime-boost strategy for immunization is utilized. Thus, in one embodiment, a nucleic acid encoding a *Lu. longipalpis* polypeptide is administered to the subject, followed by immunization with an attenuated or inactivated form of Leishmania.

The immunogenic compositions and the vaccines disclosed herein can be administered for preventative and therapeutic treatments. In therapeutic applications, compositions are administered to a subject suffering from a disease, such as *Leishmaniasis*, in a therapeutically effective amount, which is an amount sufficient to cure or at least partially arrest the disease or a sign or symptom of the disease. Amounts effective for this use will depend upon the severity of the disease and the general state of the subject's health. An effective amount of the compound is that which provides either subjective relief of a symptom(s) or an objectively identifiable improvement as noted by the clinician or other qualified observer.

Single or multiple administrations of the compositions are administered depending on the dosage and frequency as required and tolerated by the subject. In one embodiment, the dosage is administered once as a bolus, but in another embodiment can be applied periodically until a therapeutic result is achieved. Generally, the dose is sufficient to treat or ameliorate symptoms or signs of disease without producing unacceptable toxicity to the subject.

As noted above, the dosage of the composition varies depending on the weight, age, sex, and method of administration. The dosage can also be adjusted by the individual physician as called for based on the particular circumstances. The compositions can be administered conventionally as vaccines containing the active composition as a predetermined quantity of active material calculated to produce the desired therapeutic or immunologic effect in association with the required pharmaceutically acceptable carrier or diluent (for instance, carrier or vehicle). For example, about 50 μg of a DNA construct vaccine of the present disclosure can be injected intradermally three times

at two week intervals to produce the desired therapeutic or immunologic effect. In another embodiment, a about 1 mg/Kg dosage of a protein vaccine of the present disclosure can be injected intradermally three times at two week intervals to produce the desired therapeutic or immunologic effect.

A vaccine is provided herein that includes a *Lu. longipalpis* polypeptide or polynucleotide. Administration of the vaccine to a subject, such as a human or veterinary subject, results in resistance to infection with *Leishamania*. In one embodiment, the subject is a human subject. In another embodiment, the subject is a canine subject, such as a dog.

Methods and Kits for the Diagnosis of *Leishmania* Infection

It is disclosed herein that individuals who experience an anti-*Leishmania* DTH response conversion also have an increase in antibodies against *Lu. Longipalpis* polypeptide salivary proteins. Thus, the presence or absence of antibodies to *Lu. Longipalpis* polypeptide salivary proteins can be used to ascertain if a subject has a *Leishmania* infection.

A method is disclosed herein for diagnosing infection with *Leishmana* by detecting the presence of antibodies that specifically bind one or more polypeptides having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67, or a polypeptide at least 80%, at least 90%, at least 95%, or at least 99% homologous to one of these polypeptides, a conservative variant, a homolog or an immunogenic fragment of one of these polypeptides. The method can utilize a single *Lu. Longipalpis* polypeptide or a combination of these polypeptides. In certain examples, the method of diagnosis detects antibodies that specifically bind at least 3, 6, or 10 of these polypeptides, or immunogenic fragments thereof.

In one embodiment, one or more *Lu. Longipalpis* polypeptide can be bound to a solid substrate. For example, the *Lu. Longipalpis* polypeptide having an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 55, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, or SEQ ID NO: 67 can be bound to the substrate. One of more of these polypeptides can be bound to the substrate, for example at least 3, 6, or 10 of these polypeptides, or an immunogenic fragment thereof. In one example, one or more polypeptides having a sequence set forth as one of SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59 can be bound to the substrate. In another example, one or more *Lu. Longipalpis* a polypeptides having a sequence set forth as one of SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39 can be bound to the substrate. In one specific, non-

limiting example, at least six *Lu. Longipalpis* polypeptides are bound to a solid substrate, wherein each of the polypeptides comprises an amino acid sequence as set forth as SEQ ID NO: 3, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 29, SEQ ID NO: 55, or SEQ ID NO: 59, or an immunogenic fragment thereof. In another specific, non-limiting example, at least three *Lu. Longipalpis* polypeptides are bound to a solid substrate, wherein each of the polypeptides comprises an amino acid sequence as set forth as SEQ ID NO: 1, SEQ ID NO: 23, or SEQ ID NO: 39, or an immunogenic fragment thereof.

In one embodiment, two or more (for example at least 3, 6, or 10) *Lu. Longipalpis* polypeptides (or immunogenic fragments thereof) are applied to a solid substrate, for example as a series of "dots," such as in a "dot blot" assay. In another embodiment, two or more *Lu. Longipalpis* polypeptides are applied to a substrate such as in a linear array. In a further embodiment, *Lu. Longipalpis* polypeptides are applied to a membrane in a two-dimensional array. In this manner, the presence of antibodies to more than one *Lu. Longipalpis* polypeptide is assessed. Each *Lu. Longipalpis* polypeptide can be applied directly to the surface of a membrane in a single location or in a combination of locations.

The solid substrate can be a polystyrene bead, a membrane, a chip or a plate. A plastic or glass substrate can be utilized. In other embodiments, a membrane is utilized that is composed of porous materials such as nylon, nitrocellulose, cellulose acetate, glass fibers, and other porous polymers. The surface of a solid support may be activated by chemical processes that cause covalent linkage of polypeptide to the support. However, any other suitable method may be used for immobilizing a polypeptide to a solid support including, without limitation, ionic interactions, hydrophobic interactions, covalent interactions and the like. Once the polypeptide is applied to the substrate, the substrate can be contacted with a substance, such as protein-containing solution, which non-specifically saturates the binding sites thereon. Specific, non-limiting examples of a protein-containing solution include a solution made from powdered milk or serum albumin, such as bovine serum albumin.

A specimen (for example, sera, blood, plasma, urine, semen, saliva, sputum, lacrimal fluid, lymph fluid) is then added to the substrate, and the combined specimen and substrate are incubated for a sufficient time to allow specific binding. Specific binding of antibodies to the *Lu. Longipalpis* polypeptides disclosed herein, are then detected using any means known to one of skill in the art. In one embodiment, a labeled secondary antibody is used to detect the antibodies that specifically bind the *Lu. Longipalpis* polypeptides. The label can be a radiolabel (for example, ^{125}I), an enzymatic label (for example, alkaline phosphatase or horseradish peroxidase), or a fluorescent label (for example, fluoroscein isothiocyanate). Detection systems for these labels are known to one of skill in the art. Binding of the specimen, or a component of the specimen, to the *Lu. Longipalpis* polypeptide, as indicated by the presence of the marker, indicates infection with *Leishmania.*

In another embodiment, the specimen is adsorbed onto a solid substrate containing binding sites for polypeptides, such as antibody molecules. In one embodiment, the solid substrate is a polystyrene bead, a chip, a membrane or a plate. The substrate is thereafter contacted with a

substance, such as a protein-containing solution that non-specifically saturates the binding sites thereon. The substrate is then washed with a buffer. A solution of one or more *Lu. Longipalpis* polypeptides is then added to the bound specimens. In one embodiment, the *Lu. Longipalpis* polypeptide is directly labeled. The labeling of the *Lu. Longipalpis* polypeptide can be brought about by use of any marker, such as by incorporation of a radioactive isotope or group, or by coupling this component to an enzyme, a dyestuff, for example a chromophoric moiety or a fluorescent group. The enzymes of use are those which can be colorimetrically, spectrophotometrically, or fluorimetrically determined. Non-limiting examples of enzymes for use in the present invention include enzymes from the group of oxidoreductases, such as catalase, peroxidase, glucose oxidase, beta-glucuronidase, beta-D-glucosidase, beta-D-galactosidase, urease and galactose oxidase. After the labeled *Lu. Longipalpis* polypeptide is incubated with the solid substrate, any unbound labeled *Lu. Longipalpis* polypeptide is removed by washing. Bound labeled *Lu. Longipalpis* polypeptide is then detected by an appropriate assay. Binding of the labeled *Lu. Longipalpis* polypeptide to the specimen, or to a component of the specimen, is indicative of infection with *Leishmania.*

In general, the incubation steps utilized in carrying out the procedures can be performed in a known manner, such as by incubating at temperatures between about 4° C and about 25° C, for about 30 minutes to about 48 hours. Washings can be included with an aqueous solution such as a buffer, wherein the buffer is from about pH 6 to about pH 8, such as by using an isotonic saline solution of a pH of about 7.

Competitive binding assays are also of use in detecting infection with *Leishmania.* One of skill in the art, given the *Lu. Longipalpis* polypeptides disclosed herein, will readily be able to design additional assays, such as competitive binding assays, of use in detecting *Leishmania* infection.

In another embodiment, the *Lu. Longipalpis* polypeptides disclosed herein can be included in a diagnostic test kit. For example, a diagnostic test kit for detecting a *Leishmania* infection includes a solid substrate having applied thereon one or more *Lu. Longipalpis* polypeptide disclosed herein. In other embodiments, the kit includes written instructions and/or a container including a specified amount of labeled antibodies to immunoglobulins, such as IgG or IgM, or labeled secondary antibodies that bind antibodies from a species of interest. For example labeled antibodies can be provided that specifically detect dog or human immunoglobulins. The labeled antibodies can be fluorescently labeled, enzymatically labeled, or radiolabeled. Labeled antibodies used in the above-described test kits can be packaged in either solution form, or in lyophilized forms suitable for reconstitution.

In another embodiment the test kit includes a specified amount of one or more *Lu. Longipalpis* polypeptide described herein in a container, and written instructions. In one example, the *Lu. Longipalpis* polypeptide is directly labeled. In another example, the one or more *Lu. Longipalpis* polypeptide is unlabeled. If the *Lu. Longipalpis* polypeptide is unlabeled, a container can also be included with a detection reagent that specifically binds the *Lu. Longipalpis* polypeptide, such as a labeled monoclonal antibody. The kit can also optionally include a solid substrate for binding the specimen.

The above described process and test kit for detection of antibodies to the *Lu. Longipalpis* polypeptides disclosed herein can be utilized in many applications, including, but not limited to detecting *Leishmania* infection in a subject using the methods disclosed herein. The tests and kits disclosed herein can be used to detect the efficacy of a therapeutic treatment in a subject. In yet another embodiment, the tests and kits disclosed herein can also be used to assess a primary infection with *Leishmania* or to predict recovery from *Leishmania* infection by taking a body fluid from an infected subject, for example at various times following infection, and applying the above described detection procedures.

The disclosure is illustrated by the following non-limiting Examples.

EXAMPLES

Example 1
Library Construction

Sand Flies and Preparation of salivary gland homogenate (SGH). Sand fly Lutzomyia longipalpis salivary glands were obtained from colonized sand flies at Walter Reed Army Institute and at the National Institutes of Health.

Salivary glands dissected under a dissection microscope and collected in microfuge tubes in sterile phosphate buffered saline (PBS), pH 7.0 are stored in dry ice and transferred to –70° C until use.

The salivary gland of *Lu. longipalpis* is a sac-like structure consisting of a unicellular epithelium layer surrounding a large lumen (Adler and Theodor, *Ann. Trop. Med. Parasitol.* 20:109, 1926). After a blood meal, the gland total protein content decreases to half or less from its ~1μg value (Ribeiro *et al., Insect Biochem.* 19:409-412, 1989). Accordingly, most of the protein from the fly SGH must be destined for secretion. Indeed, SDS-PAGE of SGH reveals a low complexity composition consisting of ~12 major bands varying from 10-100 kD (Valenzuela *et al., J. Exp. Med.* 194:331-42, 2001). For SDS-PAGE, Tris-glycine gels (16%), 1 mm thick, and NUPAGE 12% BIS-tris gels were used (Invitrogen, Carlsbad, CA). Gels were run with either Tris-glycine or MOPS Nupage running buffer according to the manufacturer's instructions. To estimate the molecular weight of the samples, See BlueJ markers from Invitrogen (myosin, BSA, glutamic dehydrogenase, alcohol dehydrogenase, carbonic anhydrase, myoglobin, lysozyme, aprotinin, and insulin, chain B) were used.

SGH were treated with equal parts of 2X SDS sample buffer (8% SDS in Tris-HCl buffer, 0.5M, pH 6.8, 10% glycerol and 1% bromophenol blue dye). Thirty pairs of homogenized salivary glands per lane (approximately 30μg protein) were applied when visualization of the protein bands stained with Coomassie blue was desired. For amino terminal sequencing of the salivary proteins, 40 homogenized pairs of glands were electrophoresed and transferred to polyvinylidene difluoride (PVDF) membrane using 10 mM CAPS, pH 11, 10% methanol as the transfer buffer on a Blot-

Module for the XCell II Mini-Cell (Invitrogen, Carlsbad, CA). The membrane was stained with Coomassie blue in the absence of acetic acid. Stained bands were cut from the PVDF membrane and subjected to Edman degradation using a Procise sequencer (Perkin-Elmer Corp, Foster City, CA).

Salivary Gland cDNA Library Construction. Lu. longipalpis salivary gland mRNA was isolated from 80 salivary gland pairs from adult females. The Micro-FastTrack mRNA isolation kit (Invitrogen, Carlsbad, CA) was used, yielding approximately 100 ng poly (A)+ mRNA. The PCR-based cDNA library was made following the instructions for the SMART cDNA library construction kit (Clontech, Palo Alto, CA). One hundred nanograms of *Lu. longipalpis* salivary gland mRNA was reverse transcribed to cDNA using Superscript II RNase H- reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and the CDS/3' primer (Clontech, Palo Alto, CA) for 1 hour at 42° C. Second strand synthesis was performed using a PCR-based protocol by using the SMART III primer (Clontech, Palo Alto, CA) as the sense primer and the CDS/3' primer as anti-sense primer, these two primers additionally, create at the ends of the nascent cDNA SfiI A and B sites respectively. Double strand cDNA synthesis was done on a Perkin Elmer 9700 Thermal cycler (Perkin Elmer Corp., Foster City, CA) and using the Advantage Klen-Taq DNA polymerase (Clontech, Palo Alto, CA). PCR conditions were the following: 94° C for 2 minutes; 19 cycles of 94°C for 10 seconds and 68° C for 6 minutes. Double-stranded cDNA was immediately treated with proteinase K (0.8 μg/μl) for 20 minutes at 45° C and washed three times with water using Amicon filters with a 100 kDa cut off (Millipore Corp., Bedford MA). The double-stranded cDNA was then digested with Sfi I for 2 hours at 50° C (The Sfi I sites were inserted to the cDNA during the second strand synthesis using the SMART III and the CDS/3' primer). The cDNA was then fractionated using columns provided by the manufacturer (Clontech, Palo Alto, CA). Fractions containing cDNA of more than 400 base pairs (bp) were pooled, concentrated, and washed three times with water using an Amicon filter with a 100 kDa cut-off. The cDNA was concentrated to a volume of 7 μl. The concentrated cDNA was then ligated into a lambda triplex2 vector (Clontech, Palo Alto, CA), and the resulting ligation reaction was packed using the Gigapack gold III from Stratagene/Biocrest (Cedar Creek, TE) following manufacturer's specifications. The obtained library was plated by infecting log phase XL1-blue cells (Clontech, Palo Alto, CA) and the amount of recombinants was determined by PCR using vector primers flanking the inserted cDNA and visualized on a 1.1 % agarose gel with ethidium bromide (1.5 μg/ml)

Massive Sequencing of Lu. longipalpis Salivary Gland cDNA Library.

Lu. longipalpis salivary gland cDNA library was plated to approximately 200 plaques per plate (150 mm Petri dish). The plaques were randomly picked and transferred to a 96 well polypropylene plate containing 100 μl of water per well. The plate was covered and placed on a gyrator shaker for 1 hour at room temperature. Four microliters of a phage sample was used as a template for a PCR reaction to amplify random cDNAs. The primers used for this reaction were sequences from the triplex2 vector, the primers were named PT2F1 (5'- AAGTACTCT AGCAAT TGTGAGC- 3') (SEQ ID NO:71) which is positioned upstream of the cDNA of interest (5' end), and PT2R1 (5'- CTCTTCGCTATTACGCCAGCT G- 3') (SEQ ID NO:72) which is positioned downstream of the

cDNA of interest (3' end). Platinum Taq polymerase (Gibco-BRL, Gaithersburg, MD) was used for these reactions. Amplification conditions were the following: 1 hold of 75° C for 3 minutes, 1 hold of 94° C for 3 minutes and 34 cycles of 94° C for 30 seconds, 49° C for 30 seconds and 72° C for 1 minute and 20 seconds. Amplified products were visualized on a 1.1% agarose gel with ethidium bromide. Clean PCR was used as a template for a cycle sequencing reaction using the DTCS labeling kit from Beckman Coulter Inc. (Fullerton, CA). The primer used for sequencing (PT2F3) (5'-TCTCGGGAAGCGCGCCATTGTGTT - 3') (SEQ ID NO:73) is upstream of the inserted cDNA and downstream of the primer PT2F1. Sequencing reaction was performed on a Perkin Elmer 9700 thermacycler. Conditions were 75° C for 2 minutes, 94° C for 4 minutes, and 30 cycles of 96° C for 20 seconds, 50° C for 20 seconds and 60° C for 4 minutes.

After cycle sequencing the samples, a cleaning step was done using the multi-screen 96 well plate cleaning system from Millipore (Bedford, MA). The 96 well multi-screening plate was prepared by adding a fixed amount (according to the manufacturer's specifications) of Sephadex-50 (Amersham Pharmacia Biotech, Piscataway, NJ) and 300 µl of deionized water. After 1 hour of incubation at room temperature, the water was removed from the multi screen plate by centrifugation at 750 g for 5 minutes. After the Sephadex in the multi-screen plate was partially dried, the whole cycle sequencing reaction was added to the center of each well, centrifuged at 750 g for 5 minutes and the clean sample was collected on a sequencing microtiter plate (Beckman Coulter, Fullerton, CA). The plate was then dried on Speed-Vac SC 110 model with a microtiter plate holder (Savant Instruments Inc, Holbrook, NY). The dried samples were immediately resuspended with 25 µl of deionized ultrapure formamide (J.T. Baker, Phillipsburg, NJ), and one drop of mineral oil was added to the top of each sample. Samples were sequenced immediately on a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA) or stored at -30° C. The entire cDNA of selected genes was fully sequenced using custom primers using a CEQ 2000 DNA sequencing instrument (Beckman Coulter Inc., Fullerton, CA) as described above.

DNA Vaccine Construction and Description of the VR1020 Vector. The genes coding for the predicted secreted proteins were amplified from *Lu. longipalpis* specific cDNA by PCR using Platinum *Taq* polymerase (GIBCO BRL, Gaithersburg, MD) and specific primers carrying the Predicted N-terminus (Forward primer); and the stop codon (Reverse primer) of the selected cDNA.

The PCR product was immediately cloned into the custom made VR-2001-TOPO (derived fromVR1020 vector) cloning vector following manufacturers specifications (Invitrogen, Carlsbad, CA). The ligation mixture was used to transform TOP10 cells (Invitrogen, Carlsbad, CA) and the cells were incubated overnight at 37° C. Eight colonies were picked and mixed with 10 µl of sterile water. Five microliters of each sample were transferred to Luria broth (LB) with ampicillin (100 µg/ml) and grown at 37° C. The other 5 µl were used as a template for a PCR reaction using two vector-specific primers from the PCRII vector to confirm the presence of the insert and for sequencing analysis. After visualization of the PCR product on a 1.1% agarose gel, the eight PCR products were completely sequenced as described above using a CEQ2000 DNA sequencing

instrument (Beckman Coulter). Cells containing the plasmid carrying the selected *Lu. longipalpis* gene were grown overnight at 37° C on Luria broth with ampicillin (100 μg/ml), and plasmid isolation was performed using the Wizard Miniprep kit (Promega, Madison, WI). The VR-2001-TOPO (a variant of the VR1020 plasmid from Vical) plasmid contains a kanamycin resistance gene, the human cytomegalovirus promoter, and the tissue plasminogen activator signal peptide upstream of the TOPO TA cloning site. The sample that contained the sequence from the start codon to the stop codon in the right orientation and in the correct open-reading-frame following the nucleotide sequence encoding the tissue plasminogen activator signal peptide was chosen.

Plasmids were transformed into the TOP-10 strain of *E. coli* (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The transformed bacteria were grown in LB medium and the plasmid was subsequently purified using the commercial plasmid purification kit Megaprep (Qiagen, Valencia, CA). Each plasmid was named according to the name of the polypeptide. Thus pLJL34 is a plasmid encoding LJL34, and pLJM11 is a plasmid encoding LJM11 polypeptide, etc.

Study population. Sera used in the study using human subjects were obtained from an epidemiologic survey of visceral leishmaniasis (VL) in children less than 7 years of age in an endemic region of São Luiz, Maranhão State, in northeastern Brazil. During this prospective study, anti-*Leishmania* DTH and serology were performed twice a year during 1997 and 1998. Only children who had neither VL, a positive serology, nor DTH on the first survey were included in the study. None of the individuals in the data set had the disease, and all had negative responses to leishmanial antigen during the preceding 6-month period. Positivity in the anti-leishmanial tests reported here indicates a recent conversion determined by a sensitive and specific ELISA (Barral *et al., Am J Trop Med Hyg* 62:740–5, 200) and/or DTH test (Barral *et al., ibid*). To determine the cut-off value for IgG anti-*Lu. longipalpis* in ELISA assays, sera were obtained from children in the same age range from a nonendemic area. Assuming that recent seroconversion represents infection and that a positive DTH response is a marker of protection against leishmaniasis in subclinical cases, we classified children in two groups according to their anti-*Leishmania* responses: Group I, positive serology ($S^- \rightarrow S^+$) (n =15) and Group II, positive DTH ($DTH^- \rightarrow DTH^+$) (n = 15).

Anti-sand fly saliva serology. Anti-sand fly saliva serology ELISA was performed as previously described (Barral *et al., ibid*). Sera IgG subclasses were determined using anti-human IgG1, IgG3, or IgG4 alkaline-phosphatase conjugates (Sigma-Aldrich, St. Louis, MO). To determine IgE levels, sera were previously absorbed using Rheumatoid Factor. Anti-human IgE (Sigma-Aldrich, St. Louis, MO) was used in the ELISA.

Western blots. Western blots of salivary gland antigens were performed as previously described (Barral *et al., ibid*).

Statistical analysis (human studies). The non-parametric paired Wilcoxon test was used to compare levels of anti-*Lu. longipalpis* saliva antibodies in the same children at time 0 (beginning of survey) and after 6 months. P value < 0.05 was established as the significance level. Graph Pad Prism software (San Diego, CA) was used to perform the statistical tests.

Example 2

DNA and Predicted Protein Sequence Analysis.

DNA data derived from the mass sequencing project were analyzed by an in-house program written in VisualBASIC (Microsoft). This program removed vector and primer sequences from the raw sequence. Stripped sequences were compared to the NCBI non-redundant protein database using the program BlastX using the BLOSUM-62 matrix (Altschul *et al.*, *Nucleic Acids Research* 25:3389, 1997). DNA sequences were clustered by blasting the database against itself with a preselected threshold cutoff, usually $1e^{-10}$ (BlastN program) (Altschul *et al.*, *Nucleic Acids Research* 25:3389, 1997). Sequences from the same cluster were aligned using ClustalX (Jeanmougin *et al.*, *Trends Biochem. Sci.* 23:403, 1998). To find the cDNA sequences corresponding to the amino acid sequence obtained by Edman degradation of the proteins transferred to PVDF membranes from SDS-PAGE gels, a search program was written that checked these amino acid sequences against the three possible protein translations of each cDNA sequence obtained in the mass sequencing project. This was written using the same approach used in the BLOCKS (Henikoff *et al.*, *Bioinformatics* 15:471, 1999) or Prosite (Bairoch, *Nucleic Acids Res.* 19 (Suppl.):2241,1991) programs. Protein translations of the full-length clones were further processed to identify the predicted signal peptides using the Signal P program (Nielsen *et al.*, *Protein Eng.* 10:1, 1997), available online. Predicted signal peptide cleaved sites were compared to the N-terminus sequence obtained from Edman degradation of *Phlebotomus* salivary proteins. Estimation of isoelectric point and molecular weight of translated protein was performed using the DNA STAR program (DNASTAR). Full-length translated protein sequence information was compared with the non-redundant protein database of NCBI using the BLAST-P program (Altschul *et al.*, *Nucleic Acids Research* 25:3389, 1997) and searched for motifs by submitting each sequence to the electronic database.

To characterize the primary structure of the main proteins of *Lu. longipalpis* SGH, SDS-PAGE gels were transferred to PVDF membranes, and the amino terminal sequence of each cut band by Edman degradation were estimated.

In addition, the following values were ascertained:

Table 1
Protein Characteristics

Polypeptide name	Position of cleavage site	Molecular Weight (MW) of Unprocessed Protein	pI of Unprocessed Protein	Molecular Weight of Processed Protein	pI of Processed Protein
LJL34	19	31	9.14	28.9	9.1
LJL18	19	18.7	6.42	16.4	6.1
LJS193	20	34.5	6.59	32.2	6.3
LJS201	23	11.2	4.89	8.7	4.8
LJL13	19	28.7	5	26.6	4.9
LJL23	21	37.4	9.15	35.1	9.1
LJM10	19	18.8	8.73	16.7	8.6
LJL143	23	35	8.4	32.5	8.3
LJS142	20	18.9	6.43	16.7	6.5
LJL17	20	12.3	4.36	10.2	4.4
LJM06	19	18.6	8.79	16.5	8.7
LJM17	18	47.3	5.92	45.2	5.7
LJL04	17	31.1	10.1	29.3	10
LJM114	24	17	7.58	14.3	5.6
LJM111	18	45.2	4.9	43	4.9
LJM78	20	39.4	7.54	37.3	7.7
LJS238	20	6.9	7.92	4.8	6.7
LJS169	22	14.1	4.64	11.6	4.5
LJL11	24	63.4	6.49	60.8	6.7
LJL08	23	9.5	8.76	7	8.8
LJS105	19	9.5	4.85	7.4	4.7
LJL09	18	73	5.65	71.1	5.6
LJL38	20	4.8	3.66	2.5	3.3
LJM04	20	16.2	8.91	13.9	9
LJM26	17	50.7	5.77	48.8	5.8
LJS03	19	17.3	4.27	15.2	4.2
LJS192	23	12.1	4.29	9.7	4.2
LJM19	22	13.4	4.26	10.8	4.2
LJL138	19	45.9	9.42	43.8	9.5
LJL15	19	18.7	6.2	16.5	6.1
LJL91	19	18.5	5.82	16.4	5.8
LJM11	24	45.3	9.35	42.7	9.4
LJS138	20	18.5	5.88	16.2	5.5

Example 3

Antibodies against *Lu. longipalpis* saliva

It has previously been shown that sera from children living in an area endemic for VL have anti-SGS IgG antibodies that differentially recognize salivary gland antigens. Individuals with a positive anti-*Leishmania* DTH response exhibited anti-*Lu. longipalpis* saliva antibodies. A positive correlation was observed between anti-*Lu. longipalpis* saliva antibodies and anti-*Leishmania* DTH, but no correlation was observed between anti-saliva antibodies and anti-*Leishmania* serology (Barral *et al.*, *ibid*).

The change in humoral and cell-mediated anti-*Leishmania* responses in a 6-month follow up of individuals in an area endemic for VL as well as the change in anti-*Lu. longipalpis* saliva antibody responses in the same individuals was studied. Individuals ($n = 15$) who converted to positive anti-*Leishmania* DTH significantly increased their anti-*Lu. longipalpis* IgG (FIG. 1*A*; $P = 0.02$) and IgE antibody levels (FIG. 1B, $P = 0.002$). IgG1 was the principal antibody subclass involved in the increase of anti-saliva antibodies in the group converting anti-*Leishmania* DTH ($n = 15$) (FIG. 1C); no significant changes were observed in other IgG subclasses. The cut-off value for anti-*Lu. longipalpis* IgG in ELISAs was 0.045. A significant decrease in anti-saliva IgG antibody levels ($P = 0.035$) was observed in sera from children who converted their anti-*Leishmania* serology (Group I) (FIG. 1*A*). No significant changes were observed in anti-saliva IgE in Group I (FIG. 1*B*). Although IgG anti-saliva levels in Group II children decreased in the 6-month period, a significant increase in IgG4 anti-saliva was observed in this group ($P = 0.0245$; FIG. 1D).

The number and pattern of Lu. longipalpis salivary proteins recognized by the sera of individuals who converted either from $S^- \rightarrow S^+$ or from $DTH^- \rightarrow DTH^+$ was evaluated by Western blot. From seven randomly selected sera of individuals who converted their anti-Leishmania serology, two poorly recognized two different salivary proteins of 33 kDa and 200 kDa, respectively (FIG. 2A, lane 4); the remaining sera did not recognize any salivary protein at any time point. Conversely, from 13 randomly selected sera of $DTH^- \rightarrow DTH^+$ individuals, 12 recognized a variety of salivary proteins with various intensities. FIGS. 2A and 2B show the diversity of salivary antigens recognized by these sera (lanes 7-14). Additionally, sera from six $DTH^- \rightarrow DTH^+$ individuals showed an increase in the number and/or intensity of salivary proteins recognition when comparing time 0 (-) and 6 months (+) time points (FIG. 2A, lanes 7(-) and 8(+), 11(-) and 12(+), 13(-) and 14(+); FIG. 2B, lanes 11(-) and 12(+), 13(-) and 14(+), and data not shown). Some individuals in the $DTH^- \rightarrow DTH^+$ group did not show any change from time 0 to 6 months (FIG. 2A, lanes 9(-) and 10(+); FIG. 2B, lanes 7(-) and 8(+)) or did not recognize any salivary protein (FIG. 2B, lanes 9(-) and 10(+)).

The sera of the $DTH^- \rightarrow DTH^+$ individuals recognized a total of 16 different salivary proteins; however, the frequency of recognition varies among these individuals (FIG. 2C). A salivary protein of 45 kDa was recognized by 12 sera, followed by proteins of 44 and 43 and 35 kDa recognized by 8 sera (each), a protein of 17 kDa by 6 sera, and a protein of 16 kDa by 5 sera. Other salivary proteins were recognized as well but with less frequency (3 sera or less, FIG. 2C).

Thus, Group II children, who convert their anti-*Leishmania* DTH, also present an increase in anti-sand fly saliva antibodies as evidenced by ELISA and Western blot. A correlation between anti-saliva antibody titers and anti-*Leishmania* DTH has been shown (Barral *et al.*, *ibid*); the results presented herein show that development of anti-parasite DTH temporally coincides with development of anti-*Lu. longipalpis* saliva antibodies. Without being bound by theory, neutralization of sand fly salivary component(s) by antibodies or cellular response to salivary proteins allows for a more efficient mounting of an anti-*Leishmania* cell-mediated immune response, probably by developing a

Th1 response against the parasite. Sand fly saliva components, such as maxadilan, are able to impair macrophage function (Charlab *et al.*, *Proc Natl Acad Sci USA* 96:15155–60, 1999), which interferes with *Leishmania* survival and antigen presentation (Soares *et al.*, *J. Immunol.* 160:1811–6, 1998). The higher antibody levels observed in $DTH^- \rightarrow DTH^+$ individuals suggest that mounting an immune response against anti-saliva components is linked to developing cell-mediated immunity against *Leishmania*.

The results presently reported by Western blot analysis showed that individuals who converted their anti-*Leishmania* serology practically did not recognize any salivary protein whereas individuals who converted their anti-*Leishmania* DTH recognized a number of different salivary proteins. Frequency of salivary antigens recognized by these sera reveals a cluster of only a few proteins, including antigens with an approximate molecular mass of 45, 44, 43, 35, 27 and 16 kDa (FIG. 2C).

Among these antigens, the recognition of at least two salivary proteins (45 kDa and 35 kDa), represent two of the highest frequencies of recognition by human sera. Surprisingly, only two sera recognized a protein in the range of 6 kDa, the molecular weight of maxadilan (Titus and Ribeiro, *Parasitol Today* 6:157–159, 1990) suggesting that, in humans, maxadilan may not induce a strong antibody response, although it could be a strong inducer of cellular immunity.

Individuals who converted their anti-*Leishmania* cell-mediated immunity exhibited increased IgG1 and IgE levels. IgG1 has been related to a human Th1 response. Elevation of IgE antibodies suggests the development of an immediate hypersensitivity, since IgE is considered a marker of Th2-type responses. Without being bound by theory, it is likely that a mixed Th2-type (related to immediate hypersensitivity) and Th1-like response (related to DTH) against saliva components coexist in individuals who recently converted their anti-*Leishmania* DTH. In fact, this type of mixed response was reported in individuals exposed to insect bites, where the host immune response against insect saliva starts with DTH response and evolves to a predominant immediate-type hypersensitivity and finally desensitization (Melanby, *Nature.* 158, 554-555.13, 1946).

As disclosed herein, in mice, immunization using *Lu. longipalpis* salivary genes resulted in a typical DTH and/or antibody response to *Lu. longipalpis* salivary proteins (see below), suggesting that *Lu. longipalpis* bites could induce Th1 and Th2 responses in humans. Of interest, the *P. papatasii* (SP15) salivary protein responsible for the DTH response in mice is highly homologous to the SL1 protein present in *Lu. longipalpis* saliva (Charlab *et al.*, *Proc Natl Acad Sci USA* 96:15155–60, 1999). Without being bound by theory, the results presented herein suggest that a mixed anti-saliva response with both Th1 and Th2 components can help in establishing an anti-immune *Leishmania* response.

Example 4

DNA Vaccination in Mice

For genetic immunization, Swiss Webster mice were purchased from Taconic Farms. Mice were maintained in the NIAID Animal Care Facility under pathogen-free conditions. Mice were

inoculated in the right ear with 30 μg of the plasmid encoding the selected cDNA from *Lu. longipalpis* suspended in 5 μl of PBS. Each group is boosted 2 weeks later using the same regimen. Mice were challenged on the opposite ear with salivary gland homogenate of *Lu. longipalpis* and delayed type hypersensitivity (DTH) response is measured 24 hours after the injection by measuring thickness and redness of ear (++: at least 2 mice with a good DTH response, +++ : at least three mice had a good DTH response).

Table 2

DTH Response in Mice

***Lutzomyia longipalpis* salivary gland cDNA**	**DTH response (thickness and redness)**
pLJS201	-
pLJM19	++
pLJL91	-
pLJM06	-
pLJL15	+++
pLJM11	-
pLJM17	+++
pLJL11	-
pLJL08	++
pLJL18	++
pLJS142	-
pLJL13	-
pLJL34	++
pLJM111	+++
pLJL17	+++
pLJM04	-
pLJL23	++

*Delayed type hypersensitivity (DTH) response induced by injection of salivary gland homogenate on the ear of mice (group of three) previously immunized with salivary DNA vaccine. Mice were previously sensitized with specific DNA plasmids two times at two weeks interval then injected with salivary gland homogenate of the sand fly *Lutzomyia longipalpis*. DTH response was measured at 24 hours (tickness and redness of ear) after salivary gland homogenate injection. (++= at least 2 mice had good DTH response, +++ at least three mice had a good DTH response).

Example 5

Production of an Immune Response in Dogs

In a first experiment DTH (delayed type hypersensitivity) reaction is performed in dogs with natural immunity against the leishmaniasis in order to determine which *Lu. longipalpis* salivary proteins are recognized by a protective immune response. These dogs with natural immunity survived without symptoms after two years of exposure in an endemic area. In a second experiment naive dogs are immunized with the *Lu. longipalpis* salivary gland protein expressed by a plasmid in order to evaluate the capability to induce a cellular immune response measured by DTH.

Twelve dogs approximately three years old with natural immunity against Leishmaniasis are injected via an intradermal route (ID) in the back after shaving, with 100 μg of each individual plasmid suspended in 100 μl of PBS. Each plasmid is injected at a different point. The points are separated by at least 3 cm to avoid interference between DTH responses. The negative control (100 μl of buffer) is also inoculated by ID route.

The DTH response is assessed 72 hours after injection by measuring the larger diameter of the skin tumefaction area. The results are expressed as the mean value of the tumefaction area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter greater than or equal to 4 mm at 72 hours after injection.

In a second study, 10 naïve dogs 4 to 6 months old are immunized by ID injection in 10 points (100 μl per point) in the right ear with a pool of the plasmids encoding a *Lu. longipalpis* polypeptide, 100 μg for each one suspended in 1000 μl of PBS. On day 21, dogs are injected in 10 points (100 μl per point) in the left ear and in 10 points (100 μl per point) in the belly with a pool of the plasmids, 100 μg for each one suspended in 2000 μl of PBS. All dogs are challenged on day 35 by inoculation by ID route in the back (after shaving), with 100 μg of each individual plasmid suspended in 100 μl of PBS. Each plasmid is injected at a different point. The points are separated by at least 3 cm to avoid interference. As a negative control, 100 μl of buffer is inoculated intradermally. The DTH response is assessed 72 hours after challenge, by measuring the larger diameter of the skin tumefaction area. The results are expressed as the mean value of the tumefaction area for all the dogs and as a percentage of dogs having a positive DTH response. A positive DTH is a tumefaction area diameter higher or equal of 4 mm at 72 hours after injection.

The results of this study show that plasmids can induce a cellular immunity in dogs after injection, a cellular immunity reveled by a DTH response. The variation of the DTH response level can be by the variation of the expression of the insert.

It will be apparent that the precise details of the methods described may be varied or modified without departing from the spirit of the described disclosure. We claim all such modifications and variations that fall within the scope and spirit of the claims below.